Anti-Inflammatory SSTR2 Ligands

by

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Declaration

All of the work carried out in this thesis is original research carried out at The University of Warwick between October 2008 and January 2012. I declare that the material described that is not original has been identified and appropriately referenced. I certify that the material within this thesis has not been submitted for a degree at any other university.

Abstract

Broad-Spectrum Chemokine Inhibitors (BSCIs) are a novel type of anti-inflammatory drug, discovered by Fox and colleagues, which act through the receptor SSTR2. Chapter 1 consists of the story of the development of current BSCIs and a literature review of existing SSTR2 ligands.

In Chapter 2 a series of receptor probes were synthesised based on existing SSTR2 ligands, BSCIs and a hybrid of the two. Biological data were gained determining their SSTR2 binding ability and their extent of leukocyte migration inhibition.

In Chapter 3 a series of small molecules were synthesised based on the structure of highly potent BSCIs. Once again biological data were gained determining their SSTR2 binding ability and their extent of leukocyte migration inhibition.

In Chapter 4 a series of BSCIs were synthesised which contained substituted aromatic groups using an iron-cross coupling reaction. Biological data were gained to determine these compounds SSTR2 binding ability. Further iron cross-coupling reactions were carried out to determine the scope of these reactions and their applications in medicinal synthetic chemistry.

This works has gained evidence to support a split binding site theory for SSTR2. Somatostatin and BSCIs bind in slightly different area of the binding site, and through functional selectivity somatostatin structural analogues can exert an anti-inflammatory effect while somatostatin does not.

Abbreviations

acac – Acetylacetone

ad – Adamantane

ADP –Adenosine diphosphate

AMP – Adenosine monophosphate

ATP – Adenosine triphosphate

Boc – *N*-*tert*-Butoxycarbonyl

BOP – (Benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate

BSCI – Broad-Spectrum Chemokine Inhibitor

^{n}Bu – $(CH_2)_3CH_3$

^{t}Bu – $CH(CH_3)_3$

cAMP – Cyclic adenosine monophosphate

Cbz/Z – Carboxybenzyl

COX – Cyclooxygenase

Cpa – *para*-Chlorophenylalanine

d – Doublet

DABCO – 1,4-Diazabicyclo[2.2.2]octane

DAMGO – [(D-Ala-(2), N-Me-Phe-(4), Gly-(5)-ol)-Enkephalin]

DCC – *N*,*N*'-Dicyclohexylcarbodimide

DMF – Dimethylformamide

DHX – Dihydrexidine

DMAP – Dimethylaminopyridine

DMSO – Dimethylsulfoxide

DOI – (6)-1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane

dppf – 1-1'-Bis(diphenylphosphino)ferrocene

e.e. – Enantiomeric excess

ERK – Extracellular signal-regulated kinases

e.q. – Equivalent

FP – Fluorescence polarisation

FSH – Follicle stimulating hormone

GDP – Guanosine diphosphate

GH – Growth hormone

GPCR – G-protein-coupled receptor

GTP – Guanosine triphosphate

HATU – 2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate

HBTU – (2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate)

^{n}Hept – $(CH_2)_6CH_3$

^{n}Hex – $(CH_2)_5CH_3$

HOAt – 1-Hydroxy-7-azabenzotriazole

HPLC – High-performance liquid chromatography

HT – Hydroxytryptophan

IC_{50} – Half maximal inhibitory concentration

IL-8 – Interleukin-8

INF-γ – Interferon - gamma

K_i – Binding affinity of inhibitor

K_m – Binding affinity of receptor substrate

LH – Luteinizing hormone

LPS – Lipopolysaccharide

m – Multiplet

MCP-1 – monocyte chemoattractant protein-1

MIP-1α – Macrophage inflammatory protein-1α

mRNA – Messenger ribonucleic acid

MTPA – α-Methoxy-α-trifluoromethylphenylacetic acid

NI – 3-(2-Napthyl)alanine

NMM – *N*-Methylmorpholine

NMP – *N*-Methyl-2-pyrrolidone

NSAID – Nonsteroidal anti-inflammatory drugs

^{n}Oct – $(CH_2)_7CH_3$

PaI – Pyridinyl alanine

PBS – Phosphate buffered saline

^{n}Pent – $(CH_2)_4CH_3$

^{i}Pr – $CH(CH_3)_2$

^{n}Pr – $(CH_2)_2CH_3$

PyBOP – (Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate)

q – Quartet

RANTES – Regulated upon Activation, Normal T-cell Expressed, and Secreted

RPMI - Roswell Park Memorial Institute media

s – Singlet

SD - Standard deviation

SDF-1α – Stromal cell-derived factor-1-alpha

SS-14 FITC – somatostatin labelled with fluorescein isothiocyanate

t – Triplet

TFMPP – 3-Trifluoromethyl-piperazine

THF – Tetrahydrofuran

TLC – Thin layer chromatography

TMEDA – Tetramethylethylenediamine

TMU – Tetramethyl urea

TNF-α – Tumour necrosis factor-alpha

TRF – Thyrotropin releasing hormone

tRNA –Transfer ribonucleic acid

TSH – Thyroid stimulating hormone

Amino Acids

A – Ala – Alanine

C – Cys – Cysteine

D – Asp – Aspartic acid

E – Glu – Glutamic acid

F – Phe – Phenylalanine

G – Gly – Glycine

H – His – Histidine

I – Ile – Isoleucine

K – Lys – Lysine

L – Leu – Leucine

M – Met – Methionine

N – Asp – Asparagine

P – Pro – Proline

Q – Gln – Glutamine

R – Arg – Arginine

S – Ser – Serine

T – Thr – Threonine

V – Val – Valine

W – Trp – Tryptophan

Y – Try – Tyrosine

Chapter 1 – Introduction

1.1 Inflammation

Inflammation can be defined by the four latin words *calor, dolor, rubor* and *tumor* meaning heat, pain, redness and swelling. These are the physical effects seen when inflammation is present and are caused by the actions of inflammatory mediators on the local blood vessels.[1]

Inflammation is a component of the innate immune system, that is the first line of defence against a wound, injury or infection, the aim being to allow the healing process to begin by removing the harmful stimuli. Without the process of inflammation, the affected tissue would be destroyed and would fail to heal. However, inappropriate inflammation can cause or contribute to such diseases as hay fever,[2] asthma,[3] atherosclerosis,[4] rheumatoid arthritis[5] and cancer.[6]

1.1.1 The Process of Inflammation

When circulating leukocytes encounter a foreign body they engulf and destroy it. On destroying it they send out messenger molecules, including chemokines, which recruit more leukocytes to the site of infection in the process of leukocyte extravasation. Leukocyte extravasation is the movement of leukocytes out of the circulatory system to the affected area. This occurs in three steps. Initially, leukocytes are recruited to the endothelium of the affected area. Subsequently, leukocytes move between the endothelial cells and pass in to the tissue in a process called transmigration, and finally the leukocytes migrate to the site of inflammation. This accumulation of leukocytes intended to fight the infection brings about the characteristics of inflammation. Figure 1 shows healthy lung tissue compared to severely inflamed lung tissue. The purple dots are leukocytes (labelled M for

monocytes); they are confined to the tissue in the healthy lung. In the inflamed lung they have migrated to the alveoli consequently inhibiting breathing. The labels are: C capillary, E endothelial cell, P_1 and P_2 pericytes. To facilitate these processes blood vessels dilate leading to an increase in blood flow and reduction in blood flow velocity in the affected area. The endothelial cells lining the blood vessels are activated to express adhesion molecules which bind circulating leukocytes helping them migrate from the blood vessels into the tissue. This causes fluid to leak to the surrounding areas explaining the symptoms of heat, redness and swelling.[1]

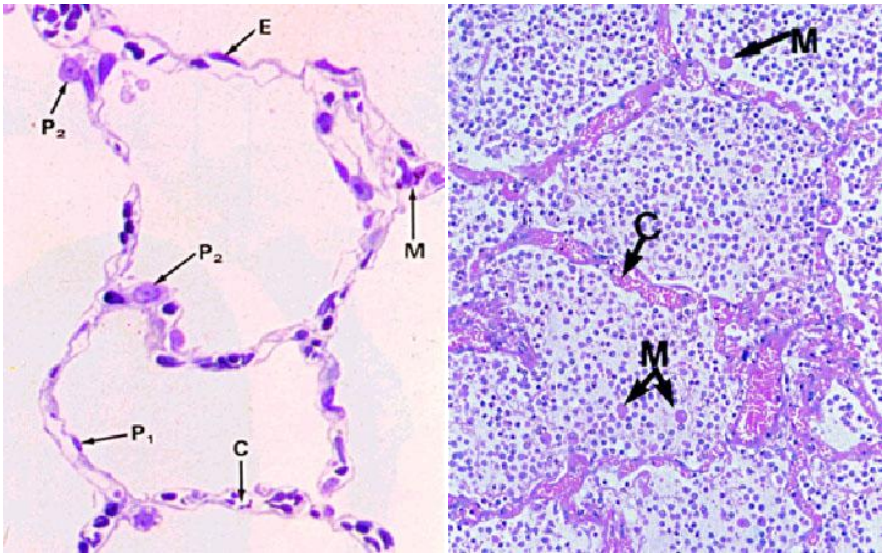


Figure 1 Healthy lung tissue[7] (left) severely inflamed lung tissue[8] (right) (taken from Young and Heath's Functional Histology and Stevens, Lowe and Young's, Basic Histopathology)

1.1.2 Current Anti-Inflammatory Drugs - NSAIDs

As previously mentioned inappropriate and chronic inflammation is a component of many diseases, consequently anti-inflammatory drugs make up about half of analgesics; they act by reducing inflammation thus reducing pain. Anti-inflammatory drugs fall into two main categories, steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) which work *via* different mechanisms. In 2010 however, three of

the top twenty best selling drugs in America were tumour necrosis factor alpha (TNF-α) inhibitors, which act by a still different mechanism. TNF-α is a cytokine which regulates inflammation. The importance of its role can be deduced by the fact it has been referred to as the 'master regulator' of inflammation.[9] All of the TNF-α inhibitors function by binding TNF-α, thereby acting as an inhibitor. Remicade[10] is a human-mouse chimeric monoclonal antibody while Humira[11] is a fully human monoclonal antibody. Enbrel[12] is a construct which is the fusion of the TNF-α receptor to the immunoglobin IgG1, and acts as a decoy receptor. It has a longer half-life than the un-fused receptor giving a longer lasting pharmacological effect.

Small molecule NSAIDs act by inhibiting the enzyme cyclooxygenase (COX) which catalyses the synthesis of the inflammatory mediators prostaglandins.[13] These are often referred to as COX inhibitors. Arachidonic acid is derived from the cellular phospholipid bilayer by the enzyme phospholipase A_2. COX catalyses the formation of PGG_2, the precursor for inflammatory mediators prostaglandins, from arachidonic acid, as illustrated in Figure 2.[14] Therefore, inhibiting COX decreases the production of prostaglandins hence reduces inflammation.[15, 16] Aspirin is one of the most famous examples of an NSAID with others including ibuprofen and naproxen.

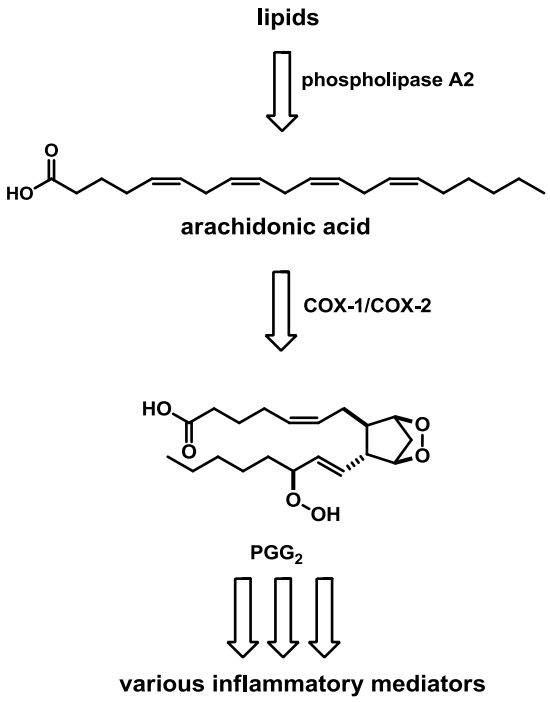


Figure 2 COX mediated production of PGG_2 from arachidonic acid

However, the NSAIDs industry is not without its problems. A major side affect of NSAIDs is stomach irritation, thought to be due to inhibiting prostaglandin. Prostaglandins play an important role in the production of gastric mucosa which has a protective effect on the stomach and has a role in preventing conditions such as stomach ulcers.[17] COX-1 expression is much higher in the gastrointestinal (GI) tract[18] and therefore selective COX-2 inhibitors have been developed to overcome this side effect. Celecoxib (**1.01**) (Figure 3) is a selective COX-2 inhibitor marketed by Pfizer which is used for the treatment of osteoarthritis and rheumatoid arthritis.[19] Vioxx (**1.02**) (Figure 3) is another COX-2 inhibitor structurally similar to Celecoxib marketed by Merck. It was withdrawn from the market in 2004 due to safety concerns causing huge losses for the company.[20]

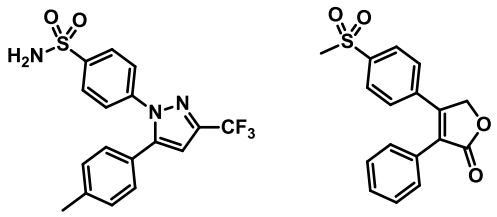


Figure 3 Celecoxib **1.01** and Vioxx **1.02**

1.1.3 Current Anti-Inflammatory Drugs - Steroids

Glucocorticoids are steroids that act by binding to the glucocorticoid receptors and therefore are often referred to as corticosteroids. They are a group of naturally occurring steroids produced in the adrenal cortex and have a variety of physiological functions including regulation of inflammation. They work by downregulating the gene expression of inflammatory mediators. The receptor is located in the cell membrane, once bound to the steroid the receptor-ligand translocates into the cell nucleus to bind to glucocorticoid response elements on the promoter region of the gene thereby regulating gene expression. Cytokines, chemokines, adhesion molecules and inflammatory enzymes, receptors and proteins are downregulated. The protein lipocortin, also known as annexin, that inhibits phospholipase A2 is also upregulated, preventing the synthesis of arachidonic acid, thereby having an anti-inflammatory affect. Corticosteroids are one of the most common drugs used in the treatment of asthma.[21, 22] Dexamethasone (**1.03**) (Figure 4), an example of a glucocorticoid drug, is 20-30 times more potent than the naturally occurring cortisol (**1.04**) (Figure 4) and it is used in the treatment of autoimmune diseases and rheumatoid arthritis. Like NSAIDs, steroidal anti-inflammatory drugs pose problems. Side effects include loss of bone density, mood changes, problems with growth and weight gain.[23] The fourth best selling drug in America in 2010 was

Advair (**1.05**) (Figure 5) which is a combination of fluticasone propionate, a corticosteroid, and salmerterol.[24]

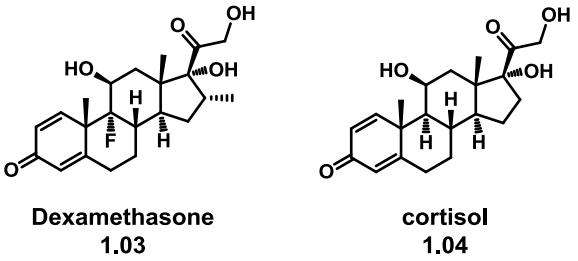


Figure 4 Dexamethasone **1.03** and cortisol **1.04**

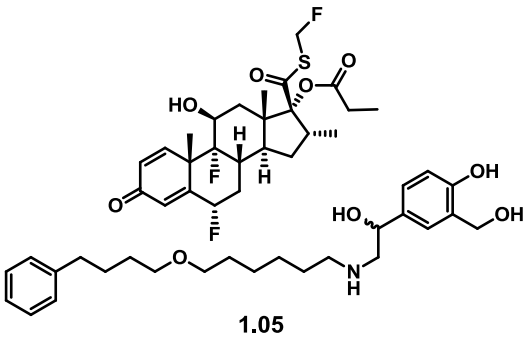


Figure 5 Advair (**1.05**)

1.1.4 Chemokines and Inflammation

The final stage of leukocyte extravasation, the migration of leukocytes to the area of inflammation, is caused by chemokines. Chemokines are a type of cytokine, named after their ability to induce chemotaxis, the directed movement of cells in response to the surrounding chemical environment. Interleukin-8 (IL-8) was discovered in 1987 and revealed the existence of this new type of cytokine.[25] Chemokines act as chemoattractants and leukocytes follow the signal of increasing chemokine concentration towards the source - the affected area. The major use of chemotaxis is to fight infection however in cases when inflammation is detrimental to the health of the individual, the inhibition of chemokines presents a mechanism by which

inflammation can be inhibited. Pathological inflammation has been shown to be reduced, or even abolished, by reducing chemokine signalling using methods such as genetic deletion or neutralising antibodies.[26] For example inflammation is reduced following treatment with non-specific pro-inflammatory stimulus, bacterial lipopolysaccharide (LPS) after mice were given neutralising antibodies against a range of chemokines.[27, 28] These results imply that chemokines are a likely pharmaceutical target in the development of anti-inflammatory agents. Chemokine regulation has to be undertaken with care, as chemokines play a vital role in defending the body against invading pathogens.[29]

1.1.5 Chemokine Structure and Chemokine Receptors

Chemokines are small proteins, being only 8-12 kDa in size.[30] There are four classes and they are divided according to the number of amino acid residues between the first and second cysteine residues closest to the *N*-terminus. These cysteine residues create the tertiary structure of the protein by forming a disulfide bonds to the third and fourth cysteine residues from the *N*-terminus. The classes are:

1) CC, where the first and second cysteine residues are adjacent. Examples include RANTES, macrophage inflammatory protein (MIP-1α) and monocyte chemotactic protein (MCP-1) which attract leukocytes such as T-cells and monocytes.

2) CXC, in which there is one residue separating the first two cysteine residues, for example IL-8 which attracts neutrophils.

3) CX_3C, in which the cysteine residues are three residues apart.

4) C, which is unique in only containing two cysteine residues.

These last two classes of chemokines are much less abundant.[31, 32]

Chemokine receptors are G-protein coupled receptors (GPCRs) which are integral membrane proteins.[30] They contain a 7-transmembrane region with the extracellular loops able to form disulfide bridges to stabilise their tertiary structure, which resembles a barrel. Ligand binding occurs within a cavity of the transmembrane domain at the *N*-terminus. The *C*-terminus is therefore intracellular and is bound to the G-proteins. GPCRs convey their signal in a guanosine triphosphate (GTP) dependent manner *via* a complex of three proteins (Gα, Gβ and Gγ) known as heterotrimeric G proteins. When guanosine diphosphate (GDP) is bound the complex is inactive and Gα binds with high affinity to Gβγ. However, when GTP is bound Gα dissociates from Gβγ and functions as a regulator or effectors protein.[33] This change in activity is thought to be due to the terminal phosphate group which, in the GTP-bound form, stabilises the Gα protein. In the GDP bound form this does not occur however the Gβγ complex stabilises it instead.[34] The exchange of GDP to GTP occurs due to the effects of a ligand binding, inducing a conformational change and causing GDP to dissociate. GTP is then able to bind, effecting another conformational change causing the GTP-Gα to dissociate and the ligand to dissociate (Figure 6). Gα has GTPase activity, which hydrolyses GTP back to GDP, thereby acting as an internal clock and switching the receptor 'off' to its original state as the Gα reassociates with the Gβγ unit.[33] GPCRs account for 80 % of the cross membrane signal transduction. They are extremely diverse, with ligands including ions, odorants, fatty acids, amino acids, neurotransmitters and polypeptides.[35]

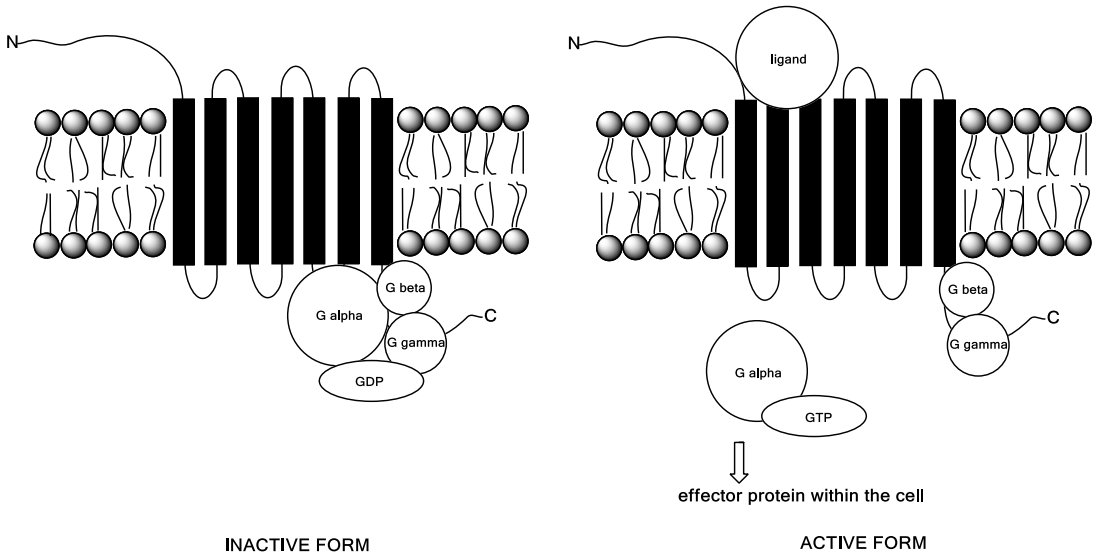
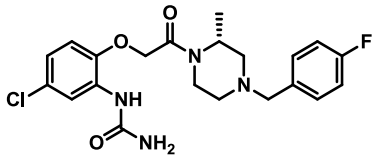


Figure 6 GPCR in the inactive and ligand bound active forms

The chemokine system is very complex; there are over 50 known chemokines and 20 receptors.[36] Different chemokines are selectively produced by a range of tissues and the receptors are expressed by a diverse array of inflammatory cells.[37] Cells can only respond if they possess a receptor that recognises the chemokine in the local environment, therefore each receptor-chemokine combination may direct a different inflammatory response and this response can be tailored by the body based on the type of injury, or threat. Receptors can only bind to one class of chemokine, but they can bind to many chemokines in that class - leading to large amounts of redundancy.[38] This leads to two approaches for pharmaceutical targets - chemokine receptor antagonists, which target single chemokine/chemokine receptor combinations, or chemokine inhibitors which target a range of chemokine/chemokine receptor combinations.

There are a number of cell migration inhibitors under development, with the majority being chemokine receptor antagonists. BX 471 (**1.06**) (Figure 7) is a potent non-peptide CCR1 antagonist which inhibits leukocyte migration and shows a 10,000-

fold selectivity for CCR1 over other receptors.[39] BX 471 has undergone clinical trials.[40] ChemoCentryx have a number of clinical candidates, including the lead candidate Traficet-EN, a CCR9 antagonist for the treatment of Crohn's disease, which has completed phase III clinical trials.[41]



1.06

Figure 7 BX 471 (**1.06**)

These examples are all based upon specific receptor-chemokine combinations. Fox and colleagues have developed a novel form of anti-inflammatory chemokine inhibitors, Broad-Spectrum Chemokine-Inhibitors or BSCIs.

1.2 Broad-Spectrum Chemokine Inhibitors - BSCIs

1.2.1 Peptide 3 and NR58, 3.14.3

BSCIs are anti-inflammatory molecules which function by inhibiting leukocyte chemotaxis induced by chemokines. The first BSCI produced by Grainger *et al* was "Peptide 3" which is comprised of residues 51-62 of the chemokine MCP-1, conserved across human, mouse and chicken species.[42] It is 12 amino acid residues long with the sequence EICADPKQKWVQ which inhibits five different chemokines: MCP-1, MIP-1α, RANTES, IL-8 and SDF-1α, with a potency of ~ 10 μM. However, it does not affect leukocyte migration caused by other chemoattractants such as *N*-formylmethionine leucyl-phenylalanine (fMLP) and tumour growth factor beta (TGF-β). NR58, 3.14.3 (**1.07**) (Figure 8) was developed from "Peptide 3" (Figure 9), being a cyclic retroinverse analogue with a similar

peptide sequence.[43] Synthesising the retroinverse analogue of a compound means changing all the amino acids to the opposite enantiomer, in this case L to D, and reversing the direction of the chain. The residues' functionalities are in the same position, but are less susceptible to proteolytic cleavage, because the proteolytic enzymes in the body are specific for L-amino acids. NR58, 3.14.3 was found to have an increased potency of over 1000 times greater than "Peptide 3".[43] The development of both of these as drugs is hindered due to poor oral availability and a short plasma half life;[44] NR58, 3.14.3 is cleared from blood plasma with a $t_{1/2}$ of less than 30 minutes following intravenous injection.[45] A solution to this problem is to synthesise smaller non-peptide analogues.

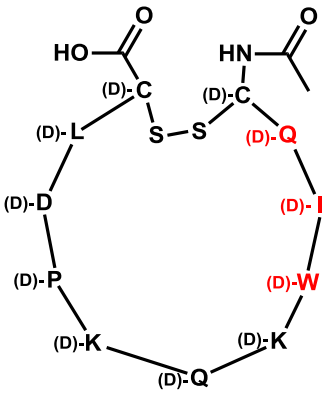


Figure 8 NR58, 3.14.3

1.2.2 Structure Activity Relationship

In order to synthesise smaller non-peptide alternatives the critical structural motif in "Peptide 3" for activity had to be determined. "Peptide 3" was divided into two hexapeptides (Figure 9). Both of these hexapeptides inhibited leukocyte migration induced by the chemokine MCP-1, but only the *C*-terminal end retained the same potency as "Peptide 3". The *C*-terminal end was then cut into two tripeptides. The

KQK tripeptide inhibited only MCP-1 while the WVQ tripeptide inhibited all five of the chemokines that "Peptide 3" did with similar potency (2-10 μM).

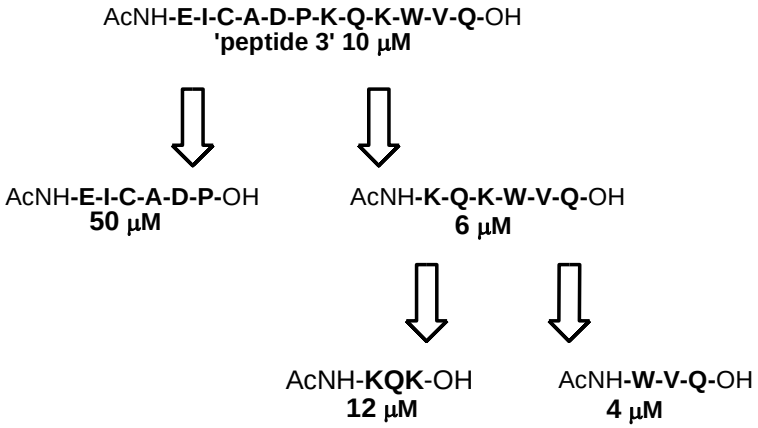


Figure 9 ED_{50} values of "Peptide 3" and derivatives

The dipeptides WV, WQ and VQ were tested but showed no activity as chemokine inhibitors so the focus was placed on the tripeptide WVQ. Replacing the tryptophan residue with any non-aromatic residues led to loss of activity, indicating the importance of a large hydrophobic group in this position. All activity was lost when replacing the glutamine residue with any other residue. In conclusion a large hydrophobic group is required at an appropriate distance from a primary amide.[45] NR58, 3.14.3 was cut down to the tripeptide WIQ, which is similar to the WVQ sequence of "Peptide 3". Isoleucine and valine both have a branched alkyl side chain, with valine just missing a CH_2 component. This fits into the conclusion drawn before that for high potency, BSCIs require a hydrophobic group and a primary amide.

1.2.3 Non-peptide BSCIs

Based on this conclusion, some potential BSCIs were synthesised. A series of glutamine peptide mimics including glutamine itself and hydrophobic groups (tryptophan mimics) were produced (Figure 10). The compound with the highest

potency of 5 nM, 2000 times higher than the next most potent compound, was NR58, 4 (**1.08**) (Figure 11). NR58, 4 is an acylaminoglutarimide with an unsaturated side chain. Compared to the (*R*)-enantiomer, the (*S*)-aminoglutarimide has a much higher potency.[45]

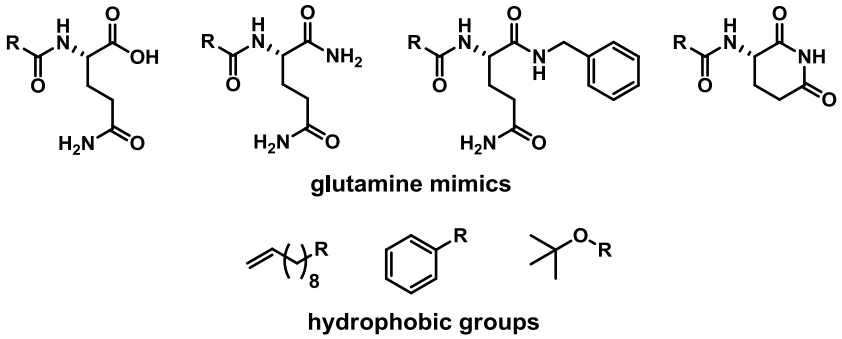


Figure 10 Q mimics and hydrophobic groups (W mimics)

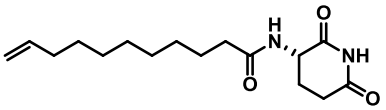


Figure 11 NR58, 4

1.2.4 NR58, 4

NR58, 4 has been shown to be an effective anti-inflammatory agent in models of acute inflammation, but less effective in models of chronic inflammation.[45] This is thought to be due to the susceptibility of aminoglutarimide rings to enzyme-catalysed metabolism.[46] A tritium labelled analogue of NR58, 4 was synthesised and injected intravenously into groups of rats, blood samples were taken at various times after injection. The total tritium content was determined by high-performance liquid chromatography (HPLC) as was the fraction of tritium in the unchanged drug. The total tritium content had a half life of 56 hours, however, only 48 hours after injection, none of the tritium was present in the form of the injected drug. This

suggests it was undergoing rapid metabolism to yield stable metabolites. Hydrolytic ring opening of glutarimide yields *N*-α-substituted glutamine and isoglutamine, which are further hydrolysed to *N*-substituted glutamic acid (Figure 12). This was confirmed by subjecting the metabolites to reverse-phase HPLC and the retention times matched those of independently synthesised products. In order to produce a molecule that retains the biological activity of the acylaminoglutarimides, but is resistant to enzyme-catalysed degradation, a series of lactam derivatives were tested. The lactam derivatives are only structurally dissimilar by the replacement of a carbonyl group with CH_2.[46]

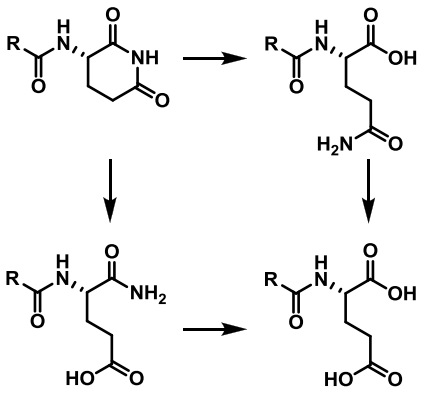


Figure 12 Hydrolytic ring opening of glutarimide ring

1.2.5 Lactam BSCIs

The 6-membered lactam equivalent of NR58, 4 (**1.09**) (Figure 13) was tested for stability. It was shown to be considerably more stable, with less than 10 % undergoing metabolism in 24 hours. This compound was not as potent as NR58, 4 but showed that these types of compounds were viable alternatives, leading to the synthesis of more lactam analogues. The 5-membered (**1.10**), 6-membered (**1.09**) and 7-membered (**1.11**) lactam equivalents of NR58, 4 were synthesised returning leukocyte migration inhibition ED_{50} values of 245 nM, 100 nM and 40 nM

respectively .[46] The 7-membered ring lactam (**1.11**) (Figure 13) was the most active, but still eight times less active than NR58, 4. By increasing the chain length the potency was increased, but solubility became a problem so unsaturated chains were produced. The result was BN83253 (**1.12**) (Figure 13) which has a potency similar to NR58, 4, 3 nM, but is relatively resistant to metabolism in serum. This compound showed greater potency than NR58, 4 when administered orally, with an effective oral availability of roughly 10 %.[46] A library of potential drug candidates was synthesised *via* the acylation of the lactams. The results showed that, like the aminoglutarimides, the (*S*)-enantiomer of the lactam was the more potent. The results also showed that the branching of the α-carbon of the side chains greatly increased their potency, which was retained even when administered orally. One of the most successful BSCI synthesised by the group (**1.13**), contains the caprolactam and a hydrophobic tertiary butyl group and has a potency of 40 pM (Figure 13).[47]

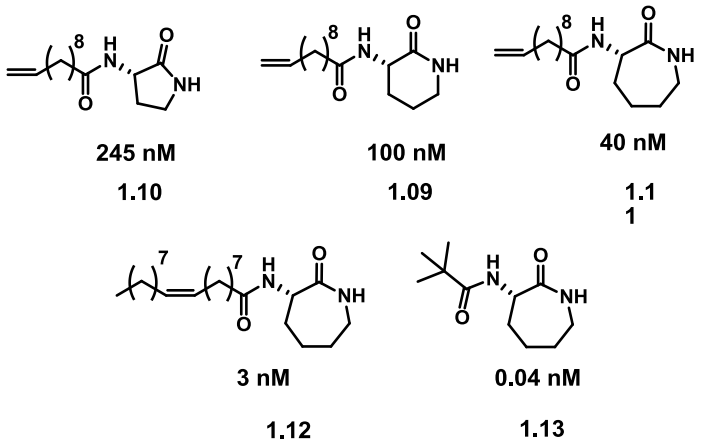


Figure 13 ED_{50}'s of compounds **1.10**-**1.13**

1.2.6 Mechanism of Action of BSCIs

The initial strategy in developing "Peptide 3" was to identify a receptor-binding antagonist, but the evidence suggests "Peptide 3" does not function through binding

to or modulating the properties of chemokine receptors. The binding of "Peptide 3" to different cell lines was studied. "Peptide 3" bound to cells expressing no chemokine receptors and when chemokine receptors were expressed at over 1 million copies per cell no additional binding was detected. "Peptide 3" had no effect on the binding of chemokines to chemokine receptors, nor did they affect the level of chemokine receptors expressed. Modifications to "Peptide 3" gave similar changes in potency in assays involving all chemokines, suggesting the target is a single receptor as opposed to a number of chemokine receptors. BSCIs only affect the migratory response aspect of chemokine signalling. They do not affect intracellular calcium ion concentrations, or downregulate chemokine receptors on the cell surface. From this it seems likely that BSCIs target a component of the chemokine signalling pathway associated with the migratory response.[48]

Through screening a large number of receptors it has been found that BSCIs in fact bind to SSTR2, a receptor classically thought of as a somatostatin receptor.[49-51]

1.3 Somatostatin and SSTR2

Somatostatin is a multi-functional peptide hormone; originally thought to be a growth hormone (GH) inhibitor. However, its functions have been found to be more diverse and can be divided into four key cellular processes such as glandular secretion, cell proliferation, smooth muscle contraction and neurotransmission.[52] It is produced by neuroendocrine cells and is involved in the integration of the nervous and hormonal systems.

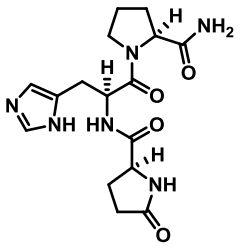
1.3.1 Discovery of Hypothalmic Releasing Factors

Somatostatin was discovered by Roger Guillemin as part of his research into brain hormone function that was to win him a share in the 1977 Nobel Prize for Physiology and Medicine.[53]

Somatostatin is one of a group of hormones known as hypothalamus releasing factors. These are hormones of hypothalmic origin and affect the release of pituitary hormones. The hypothalamus effectively links the nervous and endocrine system *via* the pituitary gland. The anatomical basis for the link between the hypothalamus and the pituitary was established by Harris and co-workers in the 1940s.[54] The hypothalamus is roughly the size of an almond and is located just above the brain stem. Hypothalmic releasing factors are produced in neuroendocrine neurons, which are in the periventricular nucleus in the third ventricle of the hypothalamus. The pituitary is roughly the size of a pea and is located below the hypothalamus in a protective bony enclosure of the skull called the sella turcica. It is comprised of three lobes: the anterior, intermediate and posterior. It is the anterior lobe which secretes hypothalmic controlled hormones. The hypophyseal portal system is a system of blood vessels which connect the hypothalamus and the pituitary. The primary plexus is composed of small capillaries which flow down the stalk of the pituitary and are distributed throughout the anterior lobe by the secondary plexus.[55]

Corticotropin-releasing factor (CRF) was the first hypothalmic hormone to be demonstrated. It stimulates the release of adrenocorticotropic hormone (ACTH) which causes the secretion of steroids by the adrenal cortex in the body's response to stress. However due to instability and difficulties with assays, not enough material was isolated to determine its structure.[55, 56]

Thyrotropin-releasing factor (TRF) (**1.14**) was the first hypothalmic releasing hormones to be isolated.[56] Initial research into these hormones was hindered by the fact that only nanogram quantities of hormone were found in each brain meaning huge numbers of brains were necessary to obtain structural data. In separate laboratories Schally and co-workers worked with primarily porcine TRF and Guillemin and co-workers worked with primarily ovine TRF. In 1968 Guillemin and co-workers isolated one mg of TRF from 300,000 sheep hypothalami.[57] The primary structure was established by mass spectrometry and it was identical to that isolated by Schally and co-workers of porcine origin.[58] TRF is a three residue polypeptide which stimulates thyroid stimulating hormone TSH and prolactin.[59] The structure (Figure 14) was determined[60] and it was successfully synthesised.[61, 62]



1.13

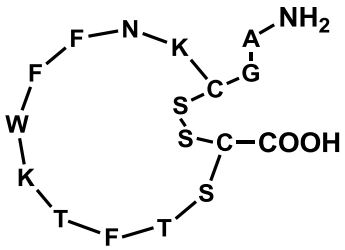
Figure 14 Thyrotropin-releasing factor (TRF)

Luteninizing hormone releasing hormone (LH-RH) or gonadotropin-releasing factor (GnRF) affects luteninizing hormone (LH) and follicle stimulating hormone (FSH).[59] In 1949 Sawyer and co-workers demonstrated the involvement of the central nervous system in the control of gonadotropin secretion.[63] The presence of LH-RH in hypothalamic extracts of rats was first detected in the early 1960s and at this point it was thought the LH releasing activity and FS releasing activity were due to two

different substances.[55] LH-RH was purified, the structure determined,[64] and it was shown to be active both *in vivo* and *in vitro*.[65]

After the discovery and isolation of TRH and LH-RH it was generally accepted that the control of the pituitary secretion of GH would also be regulated by a hypothalmic releasing factor.[66] The presence of somatostatin in the hypothalamus was first observed by Krulich and co-workers.[67] Somatostatin was isolated from 500,000 sheep hypothalami to yield 8.5 mg of somatostatin.[66] Schally and co-workers isolated somatostatin from porcine hypothalami to prove the structures were identical over both species.[68]

Somatostatin (**1.15**) (Figure 15) has the primary structure H-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH with a disulphide bond between the cysteine residues, making it a cyclic peptide. This structure was deduced by Edman degradation of the carboxymethylated peptide, the carboxymethylated tryptic digest and the chymotryptic digest.[69] The proposed structure was synthesised on solid phase and was shown to have the same properties and activity of native somatostatin.[70]



1.14

Figure 15 Somatostatin

1.3.2 Functions of Somatostatin

Somatostatin's most striking feature is the number of physiological functions it is involved in.[71, 72] As previously stated, somatostatin has been shown to not only affect GH regulation, but also glandular secretion, cell proliferation, smooth muscle contraction as well as acting as a neurotransmitter. [73] In terms of glandular secretion, somatostatin also regulates thyrotropin,[74] glucagon, insulin[75] and gastrin.[76] Smooth muscle contractions are mediated through regulation of acetylcholine, which acts as an excitatory neurotransmitter at neuromuscular junctions.[77, 78] Cells which contain somatostatin (typically neurons or endocrine-like cells) have been found in the central and peripheral nervous system, the pancreas, the gut[79] and in small numbers in the thyroid, adrenals, submandibular glands, kidneys, prostate and the placenta.[66] Consequently somatostatin has been shown to act on the brain gut, pituitary, endocrine and exocrine pancreas, adrenals, thyroids and kidneys.

1.3.4 Preprosomatostatin

Differences were noticed in the chromatographic behaviour of synthetic somatostatin compared to immunoreactive somatostatin, obtained through purification from porcine intestinal extracts. Immunoreactive somatostatin was determined to contain an *N*-terminally extended form of somatostatin. Consequently there are two active forms of somatostatin: somatostatin-14 (Figure 16) and *N*-terminally extended somatostatin-28 (Figure 17).[80]

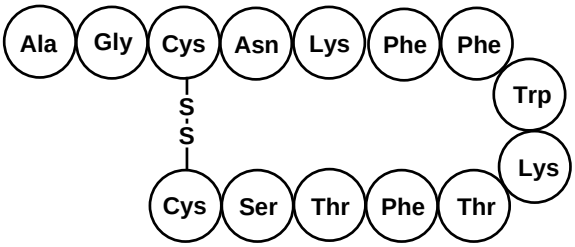


Figure 16 Somatostatin-14

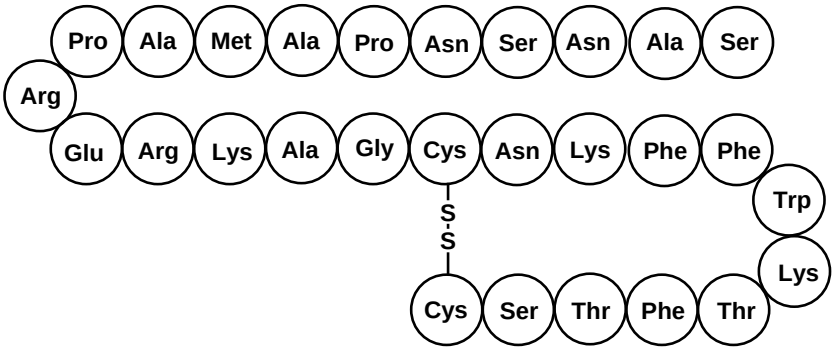


Figure 17 Somatostatin-28

Somatostatin is transcribed as inactive preprosomatostatin consisting of 116 amino acids.[81] Like other secretory proteins somatostatin is synthesised as precursors on ribosomes, then translocated into the lumen of the endoplasmic reticulum. They are then transported through budding coated vesicles through the Golgi apparatus stacks to the *trans*-Golgi apparatus network. Once in the Golgi apparatus, prosomatostatin is sorted into clathrin-coated or non-clatharin-coated vesicles ready for processing. A 24 amino acid sequence from the *C*-terminus is cleaved to yield a 92 amino acid prosomatostatin protein.[82-84] Somatostatin-14 and somatostatin-28 are both generated by endoproteolytic processing of prosomatostatin.[85-87] Processing is thought to occur by members of the subtilisin-related serine convertases family (SPCs) furin, PC1 and PC2. Cleavage occurs at the dibasic Arg-Lys cleavage site to produce somatostatin-14 and an 8 kDa peptide and is caused by the enzymes PC1 and PC2. Monobasic

cleavage at an Arg site produces somatostatin-28 and a 7 kDa peptide, this is caused by the enzyme furin.[80] In addition there is a monobasic Lys cleavage site which produces the decapeptide antrin, or prosomatostatin[1-10], a molecule without any known biological activity (Figure 18).

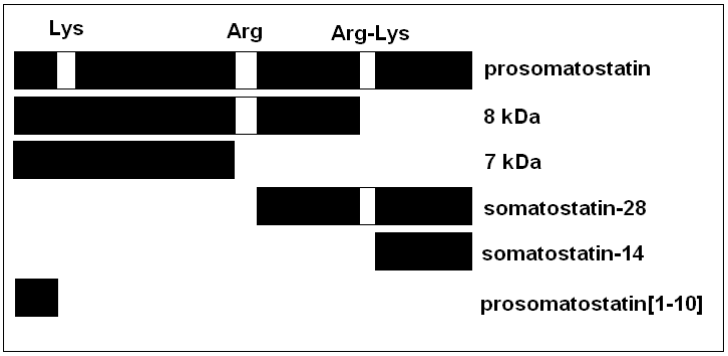


Figure 18 Schematic of mammalian prosomatostatin

Fish and other lower invertebrates have two distinct genes for somatostatin-14 and somatostatin-28. Humans have a single prosomatostatin molecule which undergoes tissue specific processing to give somatostatin-14 or somatostatin-28. The hypothalamus and cerebral cortex synthesise somatostatin in a 4:1 ratio of -14 to -28. The stomach, pancreatic islets, enteric neuron and retina produce predominantly -14 while the intestinal mucosal cells synthesise predominantly -28.[52]

1.3.5 Receptors

There are five somatostatin receptor subtypes SSTR1-5 and like chemokine receptors they are all GPCRs.[88-91] Each receptor is encoded for by separate genes on different chromosomes. Due to their similarities in structure and reactivity the receptors can be subdivided into two groups SSTR2,3 and 5 and SSTR1 and 4.[92] SSTR2 is the only receptor in which the gene coding for it has an intron, therefore it gives rise to the spliced variants SSTR2A and SSTR2B which differ in the length of the

cytoplasmic tail at the *C*-terminus. There is 39 %- 57 % sequence identity over the various receptor family members with SSTR1 and SSTR4 showing the highest sequence identity, not surprising considering they belong to the same subfamily.[88]

1.4 Somatostatin Analogues

Due to its diverse biological functions somatostatin has attracted a great deal of pharmaceutical interest. The clinical use of somatostatin itself has mainly been hampered by a short half-life, which, when in circulation, is less than three minutes. The sites of enzymatic degradation of somatostatin are shown in Figure 19.[93] A large number of analogues have been synthesised, initially to achieve greater metabolic stability than somatostatin-14. Another necessary aim is the improvement in receptor selectivity.[94]

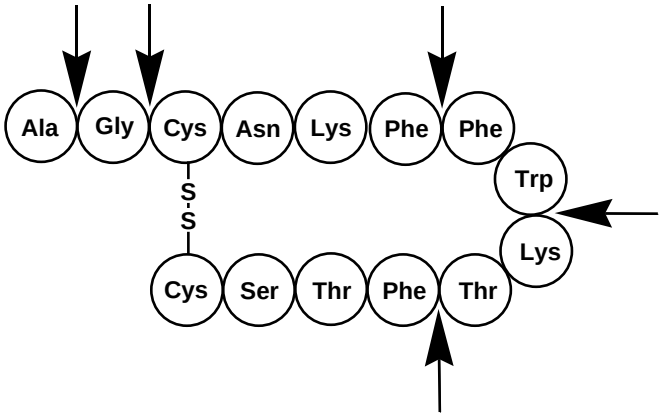


Figure 19 Sites of somatostatin susceptible to enzymatic degradation

Not long after its discovery, analogues of somatostatin were synthesised without the Ala-Gly side chain. These were shown to retain high biological activity leading to the conclusion that the cyclic structure contained the necessary binding information.[95] Initial studies were carried out to determine the minimum requirement or critical motif for activity of a somatostatin analogue. This was achieved by:

1) Systematic deletion of single residues,

2) Shortening of the *C* and *N* termini,

3) Replacing single amino acids with alanine to maintain the peptide back bone but lose the functionality,

4) Replacement of each L-amino acid with the corresponding D-amino acid, and finally,

5) Replacing residues with alternate residues.

The most significant finding was that replacing tryptophan with D-tryptophan gave an analogue with a potency of eight times higher than that of native somatostatin. This could be credited to its greater resistance to degradation being a D-amino acid, however it may be due to its greater ability to stabilise the active conformation. Complete recovery of somatostatin from the assay indicated that degradation is not an issue.[96] Another important finding was upon replacing residues Phe-(6), Phe-(7), Trp-(8), Lys-(9) or Phe-(11) with alanine, GH inhibiting potency was decreased by approximately 30-fold. The remaining residues were all expendable and replacing them with alanine had little biological effect. This study also showed the possibility of analogues which selectively inhibit one biological response over another. An analogue was synthesised with 1750 % increased inhibition of insulin over glucagon.[96]

Veber and co-workers used these findings to synthesise a cyclic hexapeptide analogue c[Pro(6)-Phe-(7)-D-Trp-(8)-Lys-(9)-Thr-(10)-Phe-(11)] called L-363,301 (**1.16**) (Figure 20), which showed a greater potency at inhibiting the release of insulin, glucagon and GH than native somatostatin.[97] This molecule was shown to

contain a βII turn around the Trp-Lys and a βVI turn around the Phe-Pro which contained a *cis*-amide bond.[98] Investigations were done into the importance of the lysine residue. Replacement with ornithine gave a 10-fold reduction in activity. Replacement with either histidine, arginine or *p*-NH_2-Phe led to a complete loss of function. Replacement with the isosteric lysine analogues thialysine and γ- and δ-fluorolysine gave comparable results to lysine. The most important factor is the precise distance of the primary amine from the peptide backbone, whilst small changes in the basicity of the residue is not important.[99] A number of similar cyclic peptides were synthesised including MK678[100] (**1.17**) (Figure 20), known as seglitide, which showed a 10-fold increase in potency. Although structurally similar, seglitide gains a hydroxyl group from the replacement of a phenylalanine with a tyrosine, yet loses a hydroxyl group in the replacement of threonine with valine. The proline is also replaced by an *N*-methylated alanine residue.

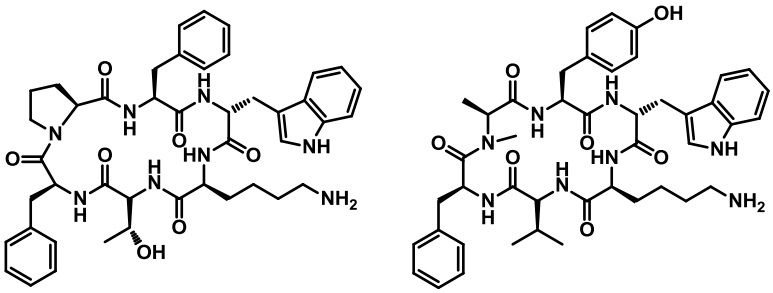


Figure 20 L-363,301 (**1.16**) and MK678 or seglitide (**1.17**)

Bauer and co-workers synthesised the most successful of the cyclic peptides - an octapeptide analogue named SMS 201-995 (**1.18**) (Figure 22). It was found to be three times more potent than native somatostatin in GH inhibition *in vitro* and more resistant to enzymatic degradation, making it 20 times more active than somatostatin

in vivo. The aim was to synthesise a compound that would inhibit GH in doses significantly less than those that would inhibit insulin. The compound must also be stable enough to inhibit GH over a therapeutically adequate time span. The starting point was a cyclic peptide Cys-Phe-D-Trp-Lys-Thr-Cys (Figure 21) cyclised by a disulphide bond between the two cysteine residues. However, this analogue had only 1/1000 of the activity of somatostatin *in vitro* or *in vivo*. Substitutions were made onto the cysteine residues at either end of the molecule. As previously mentioned Phe[6] was known to be an important residue for activity so a D-Phe residue was placed at the *N*-terminus, which also acted to hinder the disulphide bridge from enzymatic attack. The *C*-terminus was extended with a threoninol residue, which most likely mimics Thr[12] of the native somatostatin.

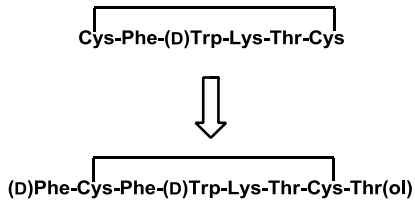
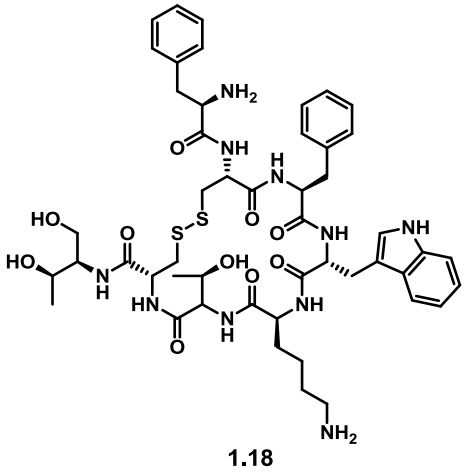


Figure 21 Cyclic hexapeptide starting point and SMS 201-995 (**1.18**)

Metabolic stability was determined by mixing the compounds with ultrafiltrate of rat kidney homogenate, as it has been shown to degrade most endogenous peptides within minutes. SMS 201-995 showed remarkable stability compared to native somatostatin[101] and with a half-life of approximately 117 minutes.[102] The disulfide bridge stabilises the biologically active sequence of Phe-D-Trp-Lys-Thr, the important β-turn is kept in over the D-Trp and Lys residues. Any modifications which change this backbone structure render the peptide inactive.[103] SMS 201-995 or octreotide, now branded as sandostatin, is sold by Novartis Pharmaceuticals as an

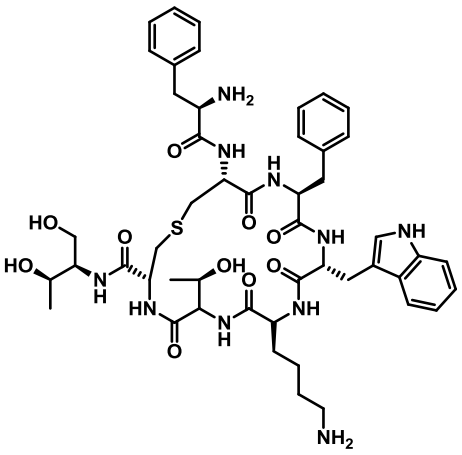
injectable drug used for the treatment of acromegaly as well as for the treatment of gastroenteropancreatic tumours and following pancreatic surgery.



1.18

Figure 22 SMS 201-995, octreotide or sandostatin (**1.18**)

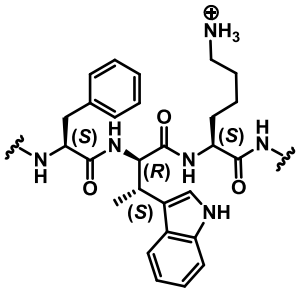
Based on the structure of sandostatin, a series of analogues were synthesised in which the disulfide bridge was replaced by a monosulfide or lanthionine bridge, for example lanthionine-octreotide (**1.19**) (Figure 23). Lanthionine bridges are components in naturally occurring molecules such as nisin[104] and epidermin.[105] They were shown to give increased selectivity to receptors SSTR2 and SSTR5 representing a good mimic for a β-turn.[106] Weaker binding meant they were not as potent as their precursor.



1.19

Figure 23 Lanthionine-octreotide (**1.19**)

Precise information regarding the structure of the cyclic peptides was obtained by the synthesis of α and β-methylated analogues of L-363,301 (Figure 20). The structure of α-methylated compounds was shown to be flat. These α-methylated compounds displayed poorer activity, indicating the folded conformation is the active one.[107] The most active analogue (**1.20**) was one which contains a β-methylated tryptophan residue, the α-centre has (*R*)-stereochemistry as in D-Trp and the β-centre was (*S*)-stereochemistry. This analogue showed improved activity compared to L-363,301 (**1.16**) (Figure 24).[108] In the active conformation the backbone amide of the tryptophan was shown to be *anti* while the lysine was *gauche* meaning the side chains were close in proximity. The phenylalanine was *anti* meaning it was also close (8-9 Å) to the tryptophan and lysine residues (Figure 25). It can be concluded that this conformation fits into the somatostatin receptor binding sites.[109] The β-methylated tryptophan residues were used later in the development of non-peptide analogues.



1.20

Figure 24 β-Methylated analogue of L-363,301 (**1.20**)

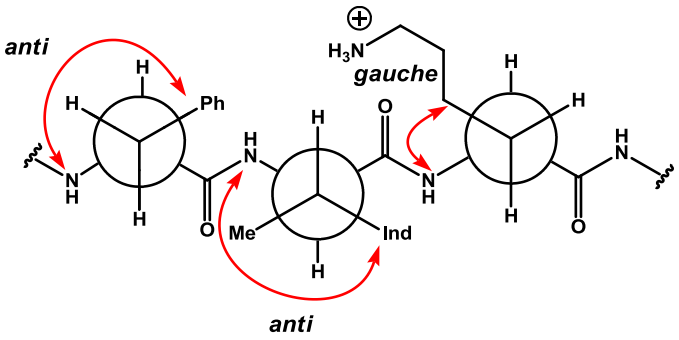


Figure 25 Newman projection of (**1.20**) β-methylated analogue of L-363,301

The synthesis of retro-inverse analogues indicated to Goodman and co-workers that the precise structure of the peptide backbone was not important - it merely held the residues in the correct orientation.[110] This was an important finding as it led to the synthesis of non-peptide and consequently small molecules analogues, more suitable for use as drugs. The challenge was to synthesise scaffolds which kept the β-turn motif found to be essential for binding, as is the case with many ligand receptor combinations.[111]

Hirschmann and co-workers replaced the peptide backbone with a β-D-glucose scaffold. Their motivation was the well defined structure and readiness for functionality the sugar scaffold provided, along with the decrease in capability of forming hydrogen bonds. The replacement of the NH by the *N*-methylated version in

cyclic peptide cyclosporin, improves its oral activity, indicating the NH bond of the peptide backbone may contribute to the bioavailability problems.[112] The β-turn was mimicked and amino acid side chains were attached through etherification reactions.[113] The resulting compounds were glucosides (Figure 26) with the side chain at positions C1, C2 and C6 mimicking D-Trp-(8), Phe-(7) and Lys-(9). They synthesised a number of compounds, for example compound (**1.21**) which bound in a dose-dependent manner. They concluded hydrogen bonding to the backbone was not necessary, the β-D-glucose scaffold could be used as a β-turn mimic and that non-peptidal peptidomimetics could act as ligands.[114, 115] A similar D-xylose mimic (**1.22**) was also synthesised (Figure 26).[116]

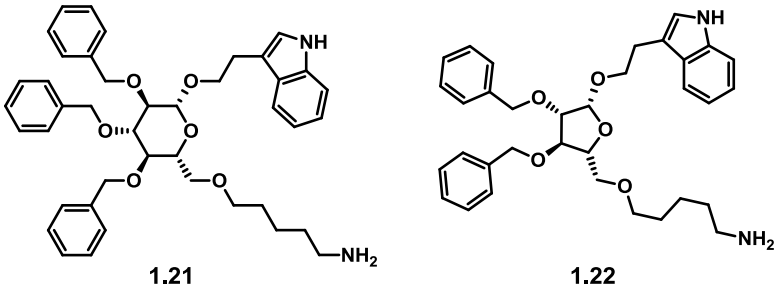
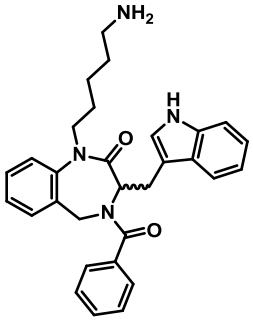


Figure 26 β-D-glucose-based (**1.21**) and a D-xylose (**1.22**)

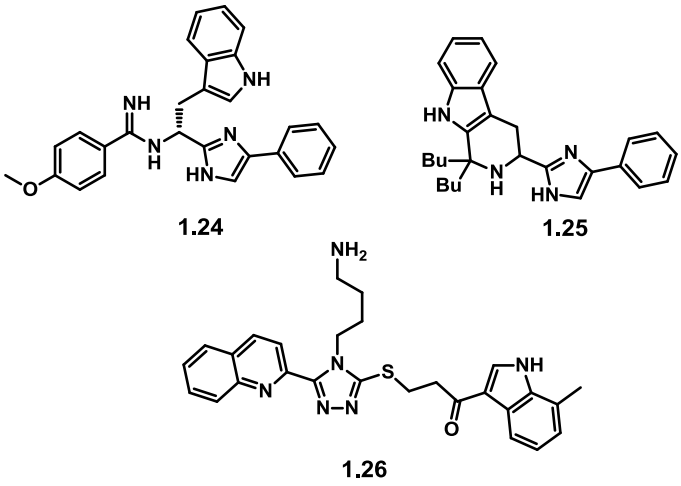
Both these compounds showed low binding affinity which was attributed to high amounts of rotational freedom in the molecules. This led to the synthesis of the 1,3,4-trisubstitued-1,4-benzodiazepin-2-one analogue **1.23** (Figure 27), being more conformationally restricted and having inherent increased bioavailability. Compared to the D-glucose and D-xylose based analogues it showed a two and three fold increase in binding respectively.[117]



1.23

Figure 27 1,3,4-trisubstitued-1,4-benzodiazepin-2-one (**1.23**)

Other non-peptide scaffolds have been reported to show selectivity. Imidazole **1.24** [118] and tetrahydro-β-carboline **1.25**[119] are known to be SSTR3 selective ligands (Figure 28). 3-Thio-1,2,4-triazoles were used as the scaffold of ligands selective for SSTR2 and SSTR5 such as the SSTR2 selective compound (**1.26**) (Figure 28).[120]



1.24 **1.25** **1.26**

Figure 28 Compounds **1.24**, **1.25** and **1.26**

The most successful ligands were probably those based on a medium ring heterocyclic backbone, which acted to keep the functional groups in the correct conformation.[121] A series of β-turn mimics were synthesised, retaining the side chain functionality and side chain 3D conformation to maintain activity.[122] These were

based on a medium ring heterocyclic scaffold that used a covalently linked thioether to replace the hydrogen bond and maintain the constrained structure (Figure 29). [123]

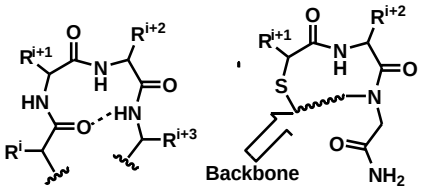


Figure 29 β-Turn motif and medium ring heterocyclic scaffold

Ellman and co-workers used these in the synthesis of some small molecule somatostatin mimics. An SSTR5 selective ligand (**1.27**) was reported with a potency of 87 nM (Figure 30).[124] Further studies to enhance its potency and selectivity were carried out. A bicyclic mimetic (**1.28**) with increased potency was synthesised (Figure 30), but it was not as selective as the parent compound for SSTR5.[125]

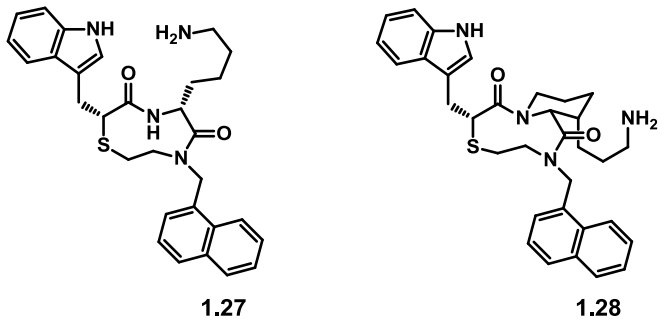
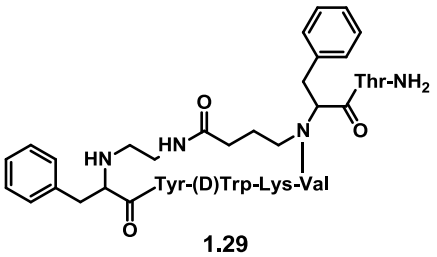


Figure 30 Compounds **1.27** and **1.28**

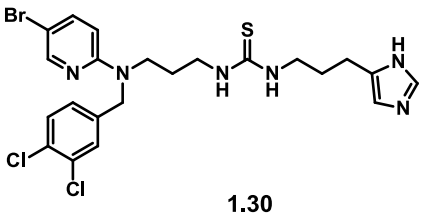
Another notable example of a ligand selective to other receptors include Kessler and co-workers' SSTR5 selective analogue, synthesised by using backbone cyclisation.[126] PTR 3046 (**1.29**) (Figure 31) is conformationally restrained by a lactam bridge and two *N*-alkylated residues, and is stable to enzymatic degradation.



1.29

Figure 31 PTR 3046 (**1.29**)

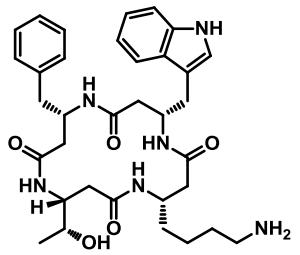
Ankersen and co-workers synthesised a series of compounds with the most potent, NNC 26-9100 (**1.30**) (Figure 32), binding to SSTR4 with a selectivity of 100-fold over other SSTR receptors. It was discovered through a screening program based on the Phe-Try-Lys-Thr motif, a scaffold containing two aromatic groups and one basic group.[127]



1.30

Figure 32 NNC 26-9100 (**1.30**)

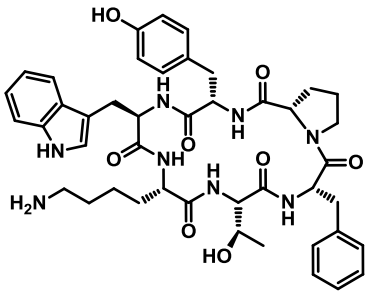
Gademann and co-workers used β-peptides as somatostatin mimics.[94] They were attracted to β-peptides for two reasons: they fold into well-defined secondary structures (for example helices, pleated sheets and turns) both in solid state and solution, and they have excellent stability against degradation (compared to natural peptides). The example shown compound **1.31** (Figure 33), is a β-peptide version of the Phe-Try-Lys-Thr β-turn motif.[128] It was shown to mimic somatostatin, albeit with a lower potency.[129]



1.31

Figure 33 β-Peptide analogue of Phe-Try-Lys-Thr motif (**1.31**)

A group at Merck synthesised the first potent non-peptide agonist which selectively binded to the SSTR2 receptor. An integrated approach of combinatorial chemistry and high-throughput receptor-binding techniques was used to rapidly identify subtype selective compounds. Using the cyclic hexapeptide L-363,377 (**1.32**) (Figure 34), was used as the basis for the probe. The chemical collection of Merck, consisting of 200,000 compounds, was searched and 75 compounds were selected for binding assays, from which a number were developed.[130]



1.32

Figure 34 L-363,377 (**1.32**)

The compounds synthesised all had small molecular weights (MW <550). L-054,264 (**1.33**) (Figure 35) is a spiro[1*H*-indene-1, 4'-piperidine] derivative; the cyclic analogue of the lysine motif gives rigidity thought to hold the amine in the correct orientation for binding. It has selectivity for SSTR2 of over 1000-fold and inhibited the release of GH with an IC_{50} of 6 nM.[131] L-054,522 (**1.34**) (Figure 35) is a

benzimidazolone derivative it contains the (2*S*,3*R*)-β-Me-D-Trp motif as described earlier and the tertiary butyl ester of lysine. It displayed increased potency and selectivity.[132] L-779,976 (**1.35**) (Figure 35), a hybrid of the two former compounds, showed 600-fold selectivity for $SSTR_2$ and inhibited GH with an IC_{50} of 0.025 nM.[130].

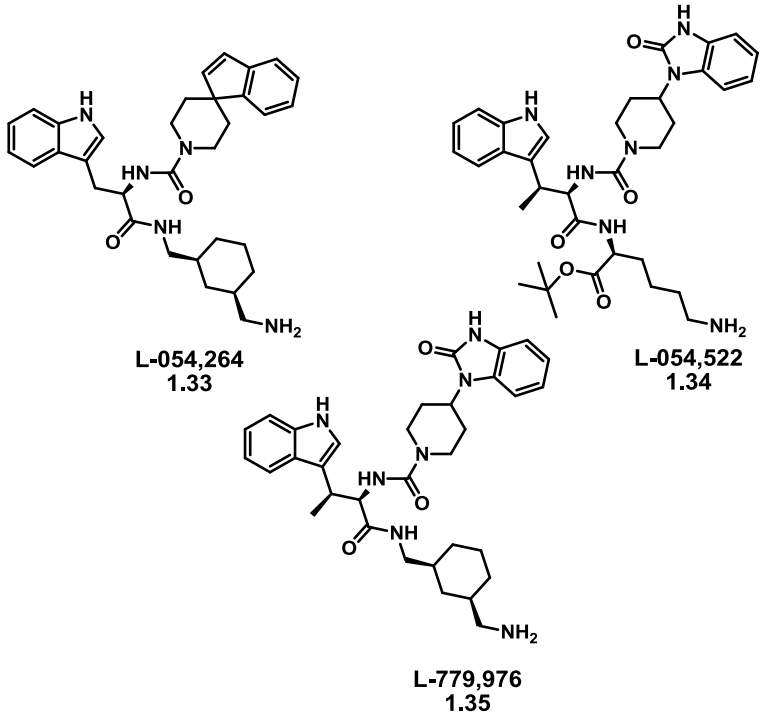
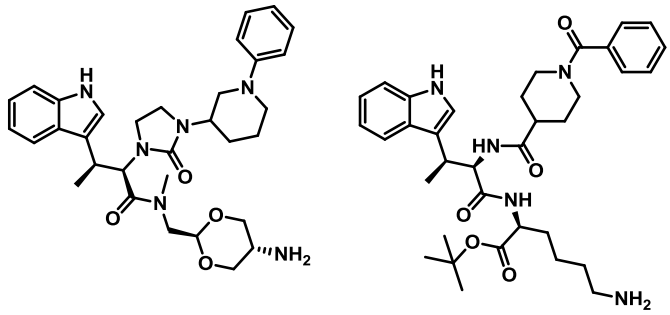


Figure 35 L-054,264 **1.33**, L-054,522 **1.34** and L-779,976 **1.35**

The main limitation of these compounds is their low oral bioavailability, so efforts were made to improve this by limiting their potential hydrogen bonding interactions. Analogues were synthesised in which the urea was *N*-methylated or replaced by a carbamate, but this led to a significant loss of potency. This was attributed to a change in conformation of the molecule which was detrimental to binding. Further analogues were synthesised with cyclisation of the urea backbone with the aim to keep it in the correct orientation for binding, but reduce its hydrogen bonding capacity. The most potent compound had a GH inhibiting IC_{50} value of 70 nM

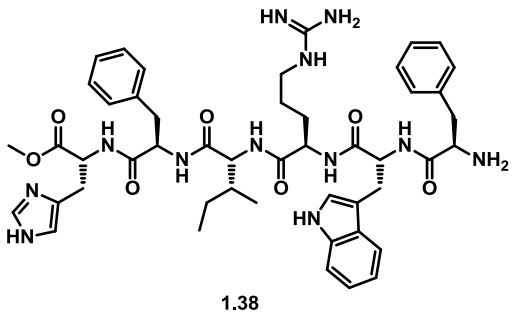
which, although not as potent as the previous molecules, it had a much improved bioavailability.[133] Through the urea cyclisation studies it was concluded that the NH of the urea was not necessary for binding . A series of *iso*-nipecotic and nipecotic acid amide structures were synthesised (**1.36**) and (**1.37**), the most potent being shown in Figure 36.[134]



1.36 **1.37**

Figure 36 Compounds **1.36** and **1.37**

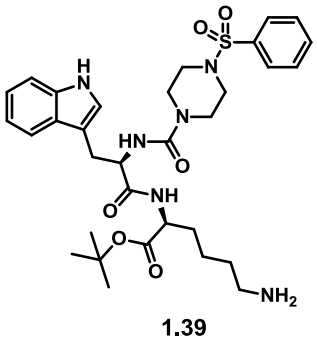
The previous compounds are all somatostatin agonists with respect to GH regulation, therefore on binding they inhibit the release of GH. Previous to this, SSTR2 antagonists have been much rarer,[135] however some do exist. The first full SSTR2 antagonist in respect to GH regulation showed binding equivalent to that of somatostatin, but without agonist qualities. In the presence of somatostatin however, it did not bind. It was a cyclic hexapeptide with the carboxy terminal cysteine a D-Cys.[136] A linear hexapeptide AC-178,335 (**1.38)** with the sequence (Ac-D-His-D-Phe-D-Ile-D-Arg-D-Trp-D-Phe-NH_2) was synthesised containing all D-amino acids and was shown to act as an antagonist *in vivo* (Figure 37).[137] Hocart and co-workers also researched peptide antagonists, again highlighting the importance of the D-Cys residue.[138, 139]



1.38

Figure 37 AC-178,335 (**1.38**)

The group at Pfizer synthesised the first small molecule SSTR2 antagonists in respect to GH regulation in the hope of upregulating GH in farm animals. It was discovered that the agonists and antagonists varied little in their structures. It is possible that small structural changes such as the sulfonamide (**1.39**) (Figure 38) might be responsible for causing the ligand to hit the antagonist binding pocket.[140]



1.39

Figure 38 Pfizer's SSTR2 antagonists (**1.39**)

1.5 The Structure of SSTR2

As previously stated somatostatin has five known receptors, but it is to SSTR2 that BSCIs bind the structure of which is shown in Figure 39.[141] Work has been carried out to determine the domains of the receptors for GH regulating ligands. MK678 (**1.17**), the selective SSTR2 agonist, was used to map the binding site by site-directed mutagenesis. It was found that the second and third extracellular loop were crucial for high affinity binding.[142] This was further investigated by the synthesis of SSTR1

and SSTR2 chimeras which showed that while the second extracellular loop was essential for SSTR1 selective binding, it was not necessary for SSTR2 selective binding. In contrast the third extracellular loop and the surrounding *trans*-membrane helices were essential for SSTR2 selective binding.[143] The sequence Phe-(294)-Asp-(295)-Phe-(296)-Val-(297) was shown to play an essential role for binding. In particular, residues Phe-(294) in *trans*-membrane helix 7 and Asn-(276) in *trans*-membrane helix-6 have been reported as key residues for binding and these interact with the residues Phe-(7)-Trp-(8)-Lys-(9)-Thr-(10) in somatostatin.[144] The folded structure of the receptor is shown in Figure 40, with the binding domain highlighted.

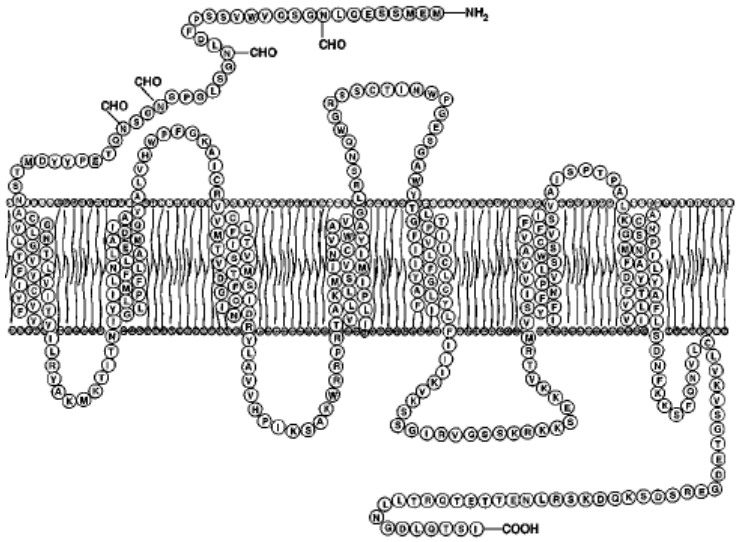


Figure 39 Structure of SSTR2 (taken from Reisine *et al*)[143]

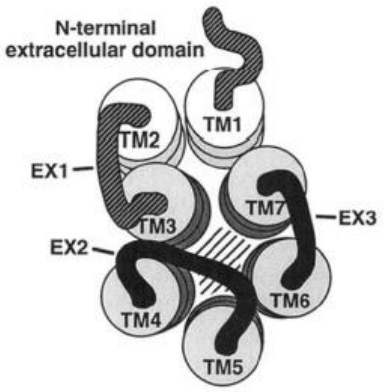


Figure 40 Folded structure of SSTR2 (taken from Lubbert *et al*)[142]

1.6 Functional Selectivity

The binding of different ligands to SSTR2 exerting a different affect to the endogenous ligand is not a novel concept.[145] It can be attributed to either functional selectivity or allosteric regulation.

Functional selectivity is a relatively new concept which goes against classical receptor ligand theory. It contrasts with the traditionally held theory associated with the idea of 'intrinsic efficacy' - the property of each molecules at the target receptor.[146] It was thought ligands could be one of the following:

1) Agonists, which bind and produce the same response as the endogenous ligand,

2) Antagonists, which bind and act as a competitive inhibitor to the endogenous ligand,

3) Inverse agonists, which bind and produce the inverse response to the endogenous ligand, or finally,

4) Partial agonists, which bind and produce a partial response of the endogenous ligand.

Each pathway that the ligand-receptor combination activates is activated in the same proportion.[147] There is mounting evidence that receptors can form ligand-specific active conformations, introducing a new concept - biased agonists theory.[148-150] Different ligands can have different efficacies regarding the different functions being performed. Due to different receptor-ligand conformations, there are different protein-protein interactions within the cell and thus a different intracellular response to each ligand. [147, 150, 151]

Mailman and co-workers discovered the functional selectivity involved in the dopamine receptor.[152] The dopamine receptor ligands dihydrexidine (DHX) and quinpirole both decrease the release from the pituitary gland of prolactin (a hormone involved in the regulation of lactation). These two ligands have different affects on striatal neurons, quinpirole being much more potent than DHX. Clarke and co-workers discovered functional selectivity at the serotonin subtype 2C ($5\text{-}HT_{2c}$) receptor. The three ligands for this receptor are 3-trifluoromethyl-piperazine (TFMPP), quipazine and (6)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI). TFMPP and quipazine both activate the inositol pathway with efficacy equal to that of the classical ligand 5-hydroxytryptamine (5-HT), but only a 60 % efficacy in activating the arachadonic acid pathway. Conversely DOI activates the arachadonic acid pathway with equal efficacy to that of 5-HT but only has 60 % efficacy in activating the inositol pathway.[147] Pharmacological, biochemical and genetic evidence have all been cited for functional selectivity by Mark von Zastrow. Pharmacological evidence is shown by differing regulation of receptor endocytosis

via clathrin-coated pits by μ-opiate receptor ligands. [(D-Ala-(2), N-Me-Phe-(4), Gly-(5)-ol)-Enkephalin] (DAMGO) and morphine were both considered full agonists, but DAMGO reduces endocytosis to a greater extent than morphine, meaning they have different levels of efficacy.[153] Biochemical studies have shown varying levels of phosphorylation of the β_2-adrenergic receptor in relation to different ligands.[154] Genetic evidence is shown by studies of active mutants of the human complement factor 5a, all of which exhibit different levels of efficacy. One mutant exhibits constant activation of G-protein mediated responses and constant endocytosis. Another mutant exhibits constant activation of G-protein responses but is only endocytosed in response to a ligand. A third mutant is activated only upon exposure to the ligand but exhibits constant endocytosis.[155]

Functional selectivity has been shown to occur at the somatostatin receptors SSTR2, SSTR4 and SSTR5.[145] Eleven somatostatin analogues were tested for their ability to inhibit cyclic AMP production and their ability to stimulate receptor internalisation. Their relative potencies for the two activities varied 10-fold and the synthetic short peptides were 5-10 times more potent at signal generation. This gave rise to the conclusion that agonists stabilise different receptor conformations with varying abilities to activate down-stream signalling pathways.[156] These findings highlighted the somatostatin receptor as a potential target for new drugs as: – *the possibility of new drugs with different pharmacological profiles at specifically targeted receptor subtypes holds great promise"*.[145] More recent evidence of functional selectivity at the SSTR2 receptor is shown by somatostatin analogues SOM230[157, 158] and KE108.[159] Both, like somatostatin, also acted as agonists for the inhibition of cAMP. Unlike somatostatin, they also acted as antagonists for stimulation of intracellular

calcium accumulation and both were partial agonists/antagonists for ERK phosphorylation.[160] Further to this, as well as showing functional selectivity in receptor signalling, they also show it in receptor regulation. Both were shown to be less able to induce receptor internalisation than somatostatin.[161]

1.6.1 Cortistatin and Functional Selectivity

Cortistatin is a neuropeptide which binds to all five somatostatin receptors.[162] It is closely related to somatostatin, yet functionally distinct, thereby displaying an example of functional selectivity. Cortistatin enhances slow wave sleep by the antagonism of the excitatory effects of acetylcholine on the cortex.[163] Cortistatin-14 has 11 of the same amino acids as somatostatin, including the motif FWKT which indicates its importance for binding. It also has two homologously placed cysteine residues responsible for the cyclic nature of the peptide. Cortistatin, like somatostatin, is coded for by a preprohormone gene, preprocortistatin a 112 amino acid length of protein. Cleavage occurs at two dibasic cleavage sites, KK and KR, to produce cortistatin-14 and cortistatin-29, analogous to somatostatin-14 and somatostatin-28. As for somatostatin, PC1 and PC2 are thought to be responsible for this cleavage as they are highly expressed in the cerebral cortex, a major site of cortistatin expression.[164]

The binding site of SSTR2 may have different conformations when somatostatin binds compared to binding with BSCIs. It is also possible that there are distinct binding sites for the individual ligands.

1.6.2 Allosteric Regulation

GPCRs are known to possess topographically distinct allosteric binding sites recognised by molecules with disparate affinities and efficacies to the orthosteric

binding site and the endogenous ligand.[165, 166] Although it is logical that the orthosteric site is the target for most drugs, it is likely this site is conserved across many subtypes, therefore the allosteric site might provide more selectivity.[167] Depending on the nature of the allosteric binding it can modulate the agonism or antagonism of the orthosteric ligand.[168] It is stated that - *"New insights into the intermolecular associability of GPCRs, with one another and with cellular accessory proteins, imply an unexpectedly broad spectrum of complexes that may presage the potential for orthosteric and allosteric ligands to attain signal pathway selectivity as a basis for new therapeutics"*.[167] The concept of bitopic ligands is relatively new.[167] A bitopic ligand is a molecule in which two topographically distinct ligand binding sites are connected together in the same molecule. Studies regarding bitopic ligand have been carried out by De Amici, Holzgrabe and colleagues on the M_2 muscarinic acetylcholine receptor. Hybrid molecules were synthesised comprising of orthosteric agonists, based on oxotremorine and a phthalimidopropane or 1,8-naphthalimido-2,2-dimethylpropane allosteric modulator. The resulting molecule displayed both allosteric and orthosteric binding properties.[169]

1.7 Somatostatin and Inflammation

As far we know, BSCIs bind to SSTR2 and exert an anti-inflammatory effect. Somatostatin, the endogenous ligand of SSTR2 has been linked with anti-inflammatory activity, although it is not often considered to be one of its major functions. It was first reported to be expressed in human immune cells by Bhathena and co-workers,[170] and subsequently it has been reported it can be synthesised and released at the site of inflammation by immune cells. Granuloma cells have been reported to express mRNA for preprosomatostatin and inflammatory mediators induce its synthesis.[171] T-cells isolated from granulomas have also been reported to

express mRNA for the receptor SSTR2.[172] Somatostatin is thought to be involved in a feedback loop regulating inflammation and T-cell function. Also involved is another neuropeptide, substance P (SP), which is associated with the processes of pain and inflammation. Somatostatin acts on T-cells to inhibit IFN-γ, a pro-inflammatory cytokine.[173] Substance P enhances INF-γ secretion[174] and downregulates somatostatin mRNA production by macrophages. Levite and co-workers reported that somatostatin controls adhesion of T-lympocytes to fibronectin, a molecule necessary for T-cell extravasation and migration.[175] However, a mechanism for its anti-inflammatory action is as yet unknown.

Somatostatin and its analogues have been reported to have anti-inflammatory effects in animal and humans. In mice treatment with somatostatin and octreotide resulted in a decreased INF-γ secretion.[173] In rats, treatment with octreotide and BIM 23014 resulted in decreased leukocyte concentration and decreased levels of local inflammatory mediators such as TNF-α and substance P.[176] In rabbits, intraarticular somatostatin was reported to reduce inflammation of chronic arthritis.[177] Reduced inflammation was reported to occur on a similar experiment with humans suffering from rheumatoid arthritis.[178-180] Particular work has focussed on rheumatoid arthritis, and SSTR2 has been reported to be expressed in synovia of affected joints.[181] Takeba and co-workers reported the inhibition of proliferation of affected synovial cells by somatostatin *in vitro*.[182] Paran and co-workers reported the results of a clinical trial in which significant clinical improvements were found in patients on treatment with the somatostatin analogue octreotide.[183] In summary, there is evidence associating somatostatin and inflammation, but little is known about the mechanism of action.

1.8 Research Hypothesis

BSCIs bind to SSTR2 and block the action of chemokines. The link between somatostatin, chemokines and inflammation has also been shown in phylogenetic relationship studies - the study of evolutionary relatedness linked to functional relatedness. It has been shown that chemokines receptors and somatostatin receptors are closely related.[184] This could potentially mean links between their intracellular signalling systems.

Because somatostatin is thought of as the endogenous ligand for SSTR2, the site to which it binds is thought of as the orthosteric site. The binding of somatostatin to this site is known to affect GH regulation. There is evidence linking somatostatin and inflammation but no known mechanism for this. BSCIs show significantly more anti-inflammatory action. The aim of this thesis is to determine whether BSCIs also bind to this orthosteric site and, through functional selectivity, cause a different intracellular effect, or whether they bind to an allosteric site, thereby changing the nature of SSTR2 agonism.

To achieve this, a library of ligands, based on current SSTR2 ligands and hybrid ligands (which in addition contain the acylaminolactam BSCI structure), were synthesised (as shown in section 2.1). These were subsequently tested in two functional assays, an SSTR2 binding assay and a leukocyte migration assay.

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Chapter 2 - SSTR2 Ligands, BSCIs and Hybrids

2.1 Introduction

The aims of this thesis are to determine by which mechanism BSCIs act at SSTR2 whether it be through functional selectivity or allosteric regulation. As a starting point a catalogue of molecules was synthesised in order to both probe the receptor SSTR2, and to gain information on the critical motifs responsible for activity for both SSTR2 binding and BSCI activity (see section 1.4 and 1.2.1). The molecules were based on current SSTR2 ligands such as compound **1.39**, current BSCI compounds such as lactam **1.13** (Figure 41) and on hybrid structures of the two classes.

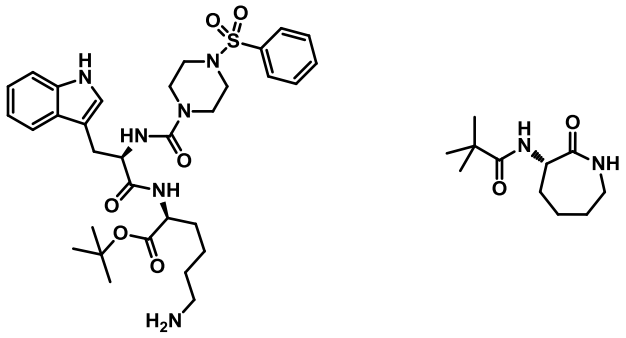


Figure 41 Current SSTR2 ligand by Pfizer[140] and current BSCI[47]

Current SSTR2 ligands are based on the tripeptide sequence KWF, the critical motif of activity for somatostatin (Figure 42).[132] In comparison, current BSCIs are based on the tripeptide WxQ, in which x is a hydrophobic amino acid. This sequence was derived from "Peptide 3", the initial compound shown to have BSCI activity, and is also present in NR58, 3.14.3, a second generation BSCI. The hydrophobic residue is valine in "Peptide 3" and isoleucine in NR58, 3.14.3, though activity is retained

when phenylalanine is substituted for these residues.[45] In addition both "Peptide 3" and NR58, 3.14.3 contain a lysine residue preceding this tripeptide sequence. The similarities in these two sequences are apparent and indicate why they both bind the receptor SSTR2 (Figure 42).

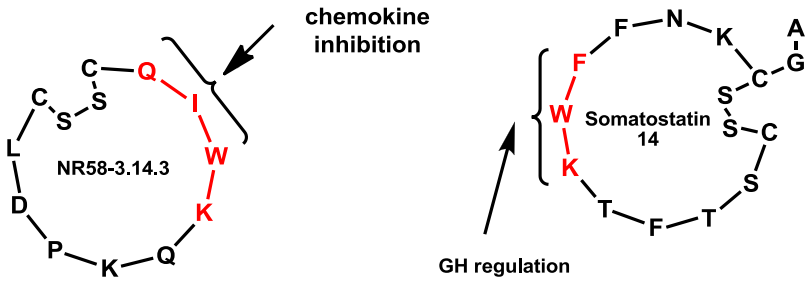


Figure 42 Critical motifs for activity for BSCI NR58, 3.14.3 and somatostatin

Molecules based on the KWF sequence or the hybrid KWFQ sequence were synthesised with a lactam representing the glutamine residue (Figure 43). Molecules containing both 6-membered and 7-membered lactams have been synthesised, as have simplified molecules, replacing the lactam moiety with a methyl substituent in both the *para* and *meta* positions. The lysine and tryptophan components of the molecules have been gradually reduced and eventually removed altogether over the sequence. The molecules synthesised can be split into five groups represented by compounds **2.01**-**2.09** (Figure 43):

1) lysine-tryptophan-phenylalanine (KWF analogues, e.g. compound **2.01**-**2.03**)

2) tryptophan-phenylalanine (WF analogues, e.g. compounds **2.04**),

3) lysine-tryptophan-phenylalanine-glutamine (KWFQ analogues, e.g. compounds **2.05**-**2.07**),

4) tryptophan-phenylalanine-glutamine (WFQ analogues, e.g. compound **2.08**),

5) phenylalanine-glutamine (FQ analogues, e.g. compound **2.09**).

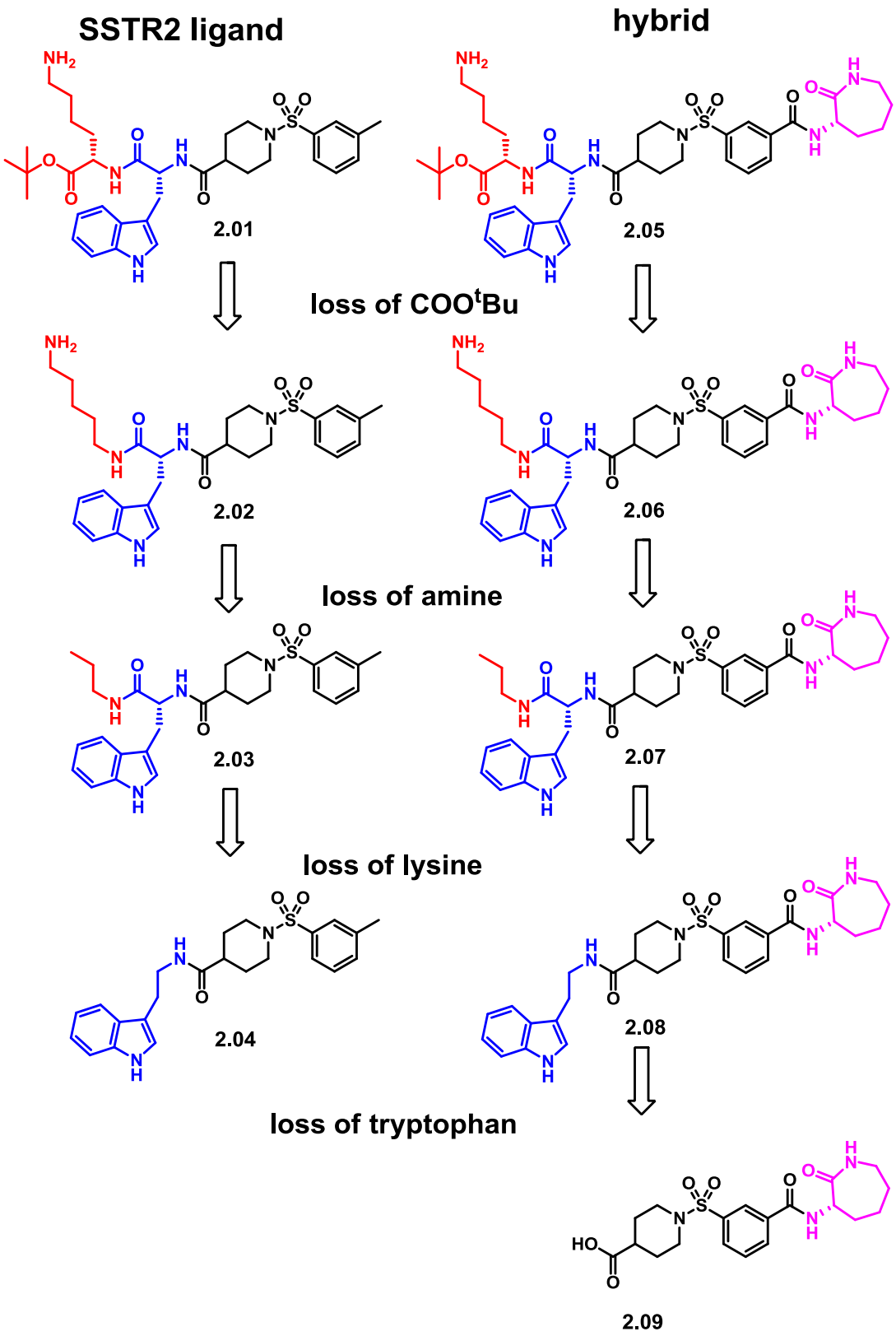


Figure 43 Catalogue of molecules synthesised; K mimics (red), W (blue), F (black) and Q (pink)

2.2 Non-Lactam Containing Molecules

The compounds based on current SSTR2 ligands were either KWF or WF mimics. Disconnections were made between the lysine and tryptophan motifs, and the tryptophan and phenylalanine motifs (Figure 44). Each amino acid motif was synthesised separately before being coupled together. This synthetic sequence is similar to that used by Merck in the synthesis of related SSTR2 ligands.[132]

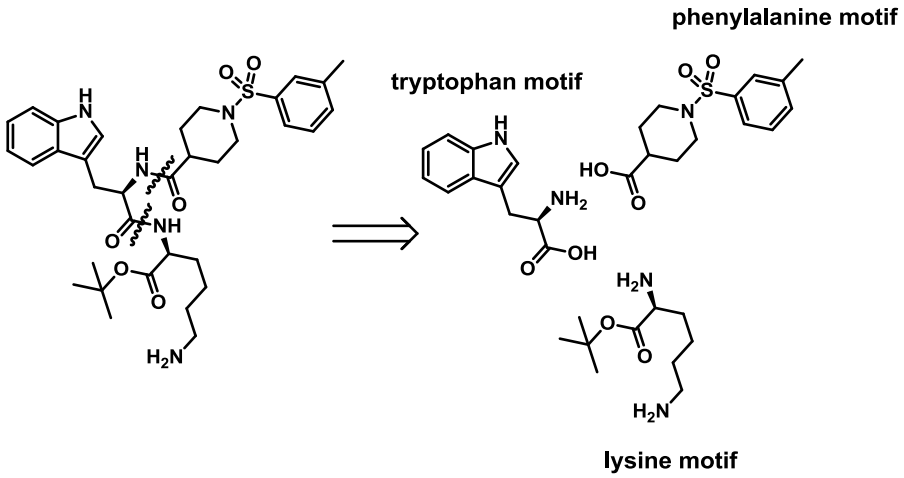
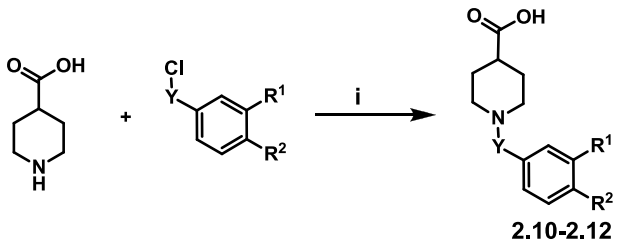


Figure 44 Disconnection of **2.01** used in the synthesis of SSTR2 like ligands

2.2.1 Phenylalanine Mimics

Three phenylalanine mimics, acids **2.10**-**2.12**, were synthesised by coupling isonipecotic acid to commercially available acid chlorides, using either benzoyl chloride or the sulfonyl chlorides *para*-toluenesulfonyl chloride and *meta*-toluenesulfonyl chloride (Scheme 1). These biphasic amide-forming reactions are examples of a Schotten-Baumann reaction.[185]

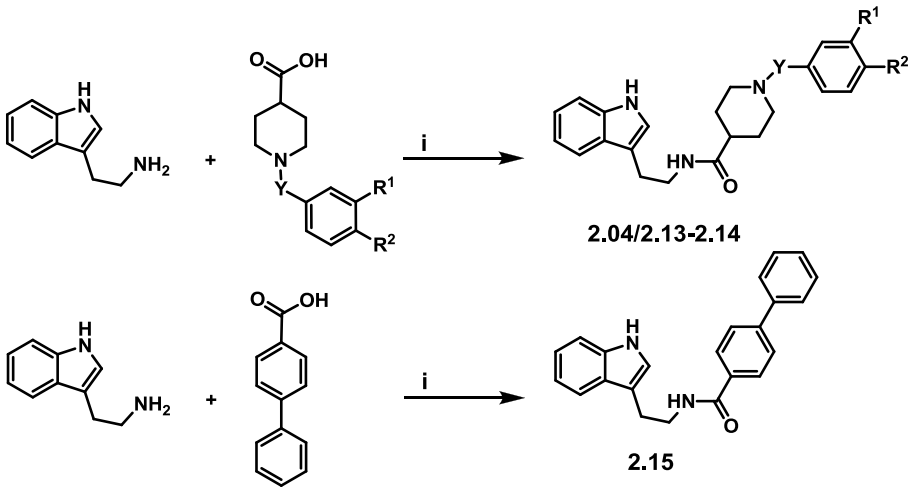


(2.10): Y=SO_2, R^1=Me, R^2=H, (76 % yield), **(2.11)**: Y=SO_2, R^1=H, R^2=Me, (84 % yield)
(2.12): Y=CO, R_1=H, R_2=H, (67 % yield)
Reagents; (i) THF/H_2O, 3 eq. Et_3N

Scheme 1 Synthesis of compounds **2.10**-**2.12**

2.2.2 Phenylalanine-Tryptophan Mimics

Compounds **2.10**, **2.11** and **2.12** and 4-biphenyl carboxylic acid, another phenylalanine mimic, were coupled to the commercially available tryptamine. This furnished the smallest of the SSTR2 type ligands, the WF mimics (**2.04** and **2.13-2.15**). The reaction was carried out in dichloromethane with 2-(1H-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate methanaminium (HATU) as a coupling agent and triethylamine as a base (Scheme 2).



(2.04): Y=SO_2, R^1=Me, R^2=H, (32 % yield),
(2.13): Y=SO_2, R^1=H, R^2=Me, (48 % yield),
(2.14): Y=CO, R^1=H, R^2=H (48 % yield), **(2.15):** (55 % yield)
Reagents; (i) CH_2Cl_2, HATU, 3 eq. Et_3N

Scheme 2 Synthesis of compounds **2.04** and **2.13**-**2.15**

2.2.3 The Use of HATU

Formation of an amide requires activation of the carboxylic acid, usually by making it into an ester with a good leaving group. There are a number of peptide coupling reagents, including carbodiimides such as *N*,*N*'-dicyclohexylcarbodiimide (DCC) and uronium or phosphonium reagents such as benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (BOP), benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) and HATU.[186] Carbodiimides generate by-products that are difficult to remove from the product, for this reason HATU was the chosen peptide coupling reagent. Crystal structures and NMR data have shown that HATU exists in the guanidinium form (also known as the *N*-form), unlike the analogous structure of BOP and PyBOP which exist in the uronium form (also known as the *O*-form) due to the high stability of the P-O bond (Figure 45).[187]

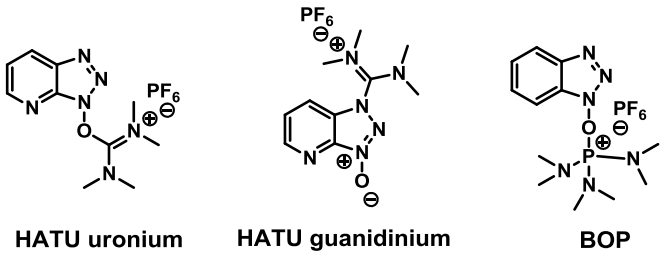


Figure 45 *O*-form and *N*-form of HATU and *O*-form of BOP

In the *N*-form of HATU there is more double bond character between the nitrogen of the triazole ring and the carbon with the two $N(CH_3)_2$ groups, best represented by the right hand resonance form of Figure 46. This restricts rotation around this bond, meaning the $N(CH_3)_2$ protons are inequivalent and the 12 protons of the dimethyl groups appear as two singlets for six protons each at 3.00 and 3.40 ppm in the ^1H NMR.[187] In contrast, the *O*-form of HATU shows one singlet at 3.24 ppm that

integrates for all 12 protons.[187] This is due to higher double bond character between the carbon with the two $N(CH_3)_2$ groups and the nitrogen of the $N(CH_3)_2$ group, best represented by the left hand resonance form of Figure 46. This leads to a greater degree of rotation around the oxygen bonds, so the twelve $N(CH_3)_2$ protons all appear equivalent, and hence appear as only one singlet in the ^1H NMR. The reason the oxygen to carbon bond displays less double bond character compared to the carbon to nitrogen bond in the *N*-form is because oxygen is more electronegative, and hence an oxygen cation is less stable than a nitrogen cation. Experimentally, the ^1H NMR of the HATU used showed two singlets corresponding to six protons at 3.39 and 3.04 ppm, confirming it was the *N*-form. The *N*-form also shows a characteristic IR band at 1664-1675 cm^{-1} while the *O*-form shows a characteristic IR band at 1709-1711.[187] The HATU used showed a band at 1664 cm^{-1}, further confirming the structure as the *N*-form.

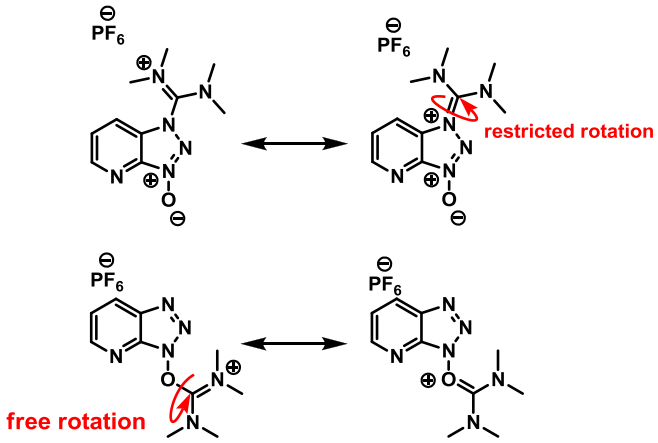
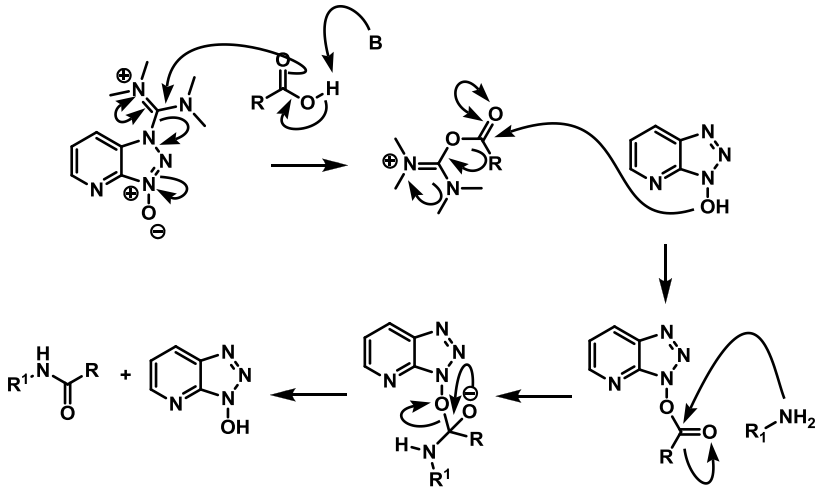


Figure 46 Resonance structures of the *N*- and *O*-form of HATU, *N*-form favours the structure on the right, *O*-form favours the structure on the left

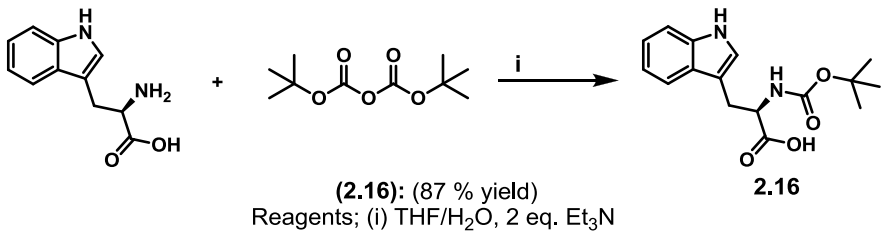
HATU reacts with the carboxylic acid to form an active ester and tetramethyl urea; on addition of the amine it reacts with the active ester to form the amide bond (Scheme 3).



Scheme 3 Mechanism of HATU forming an active ester[186]

2.2.5 Lysine-Tryptophan Mimics

The second disconnection in the synthesis (Figure 44) is between the tryptophan and lysine motifs to create the KWF mimics. To allow the coupling of the carboxylic acid functionality of tryptophan to the lysine motif, the tryptophan amine was protected with di-*tert*-butyldicarbonate (Boc protected) to give acid **2.16** (Scheme 4).



Scheme 4 Synthesis of compound **2.16**

Five different commercially available amines (**2.17-2.21**) were coupled to acid **2.16** to give compounds **2.22-2.26**. The amines were propylamine **2.17**, *N*-Z,1,4-

diaminobutane **2.18**, *N*-Z,1,4-diaminopentane **2.19**, *N*-Z,1,4-diaminohexane **2.20** and H-lysine(Z)-O^{t}Bu **2.21**. The last four of that list are mono-carboxybenzyl- (Cbz) protected diamines. These reactions were performed using HATU as a coupling agent and triethylamine as a base (Scheme 5).

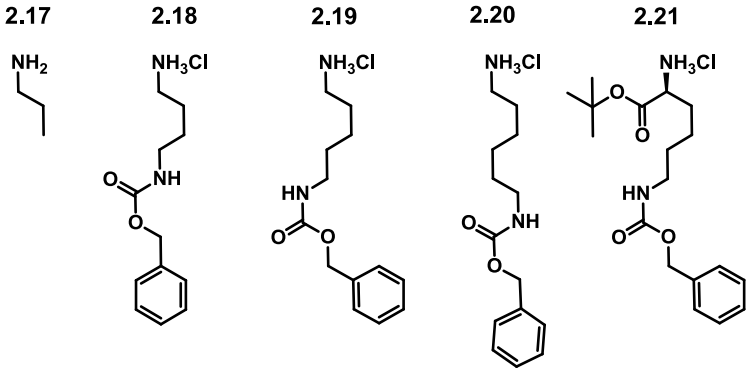
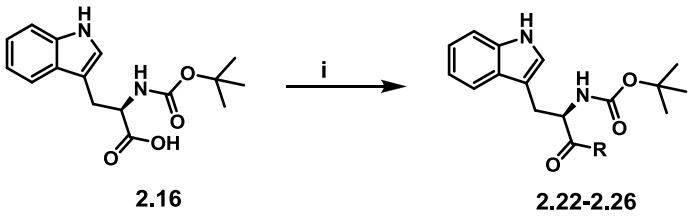


Figure 47 Amines or their hydrogen chloride salts **2.17-2.21**

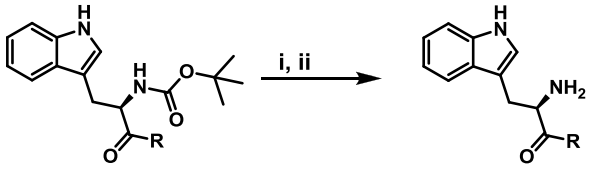


(2.22): R=**2.17**, (79 % yield), **(2.23):** R=**2.18**, (67 % yield), (**2.24):** R=**2.19**, (59 % yield), **(2.25):** R=**2.20**, (10 % yield), **(2.26):** R=**2.21**, (52 % yield)
Reagents; (i) CH_2Cl_2, HATU, 3 eq. Et_3N and R

Scheme 5 Synthesis of compounds **2.22**-**2.26**

2.2.6 Removal of the Boc Group

The next stage was to deprotect the tryptophan amines by removal of the Boc groups to allow further coupling to the phenylalanine mimics. Boc groups are removed by acid hydrolysis. The reactions were performed by dissolving the compounds in methanol and adding three equivalents of methanesulfonic acid (Scheme 6). The free amines (**2.27**-**2.31**) were provided by addition of triethylamine and reacted without further purification.



2.22-2.26 **2.27-2.31**

(2.27): R=**2.17**, **(2.28):** R=**2.18, (2.29):** R=**2.19**,
(2.30): R=**2.20**, **(2.31):** R=**2.21**
Reagents; (i) CH_3OH, 3 eq. CH_3SO_2OH, (ii) 5 eq. Et_3N

Scheme 6 Compounds **2.27-2.31**

The presence of the tertiary butyl ester in **2.26** (Figure 49) posed a problem, as this must be left unaffected while the structurally similar tertiary butyl carbamate group is removed. This is possible because of the increased basicity of the Boc group, due to the conjugation of the lone pair from the nitrogen in the amide bond, which stabilises the protonated carbonyl oxygen. This stabilisation of the protonated amide, and subsequent stabilisation of the transition state, means the activation energy for Boc removal is lower than for the tertiary butyl ester removal (Figure 48).

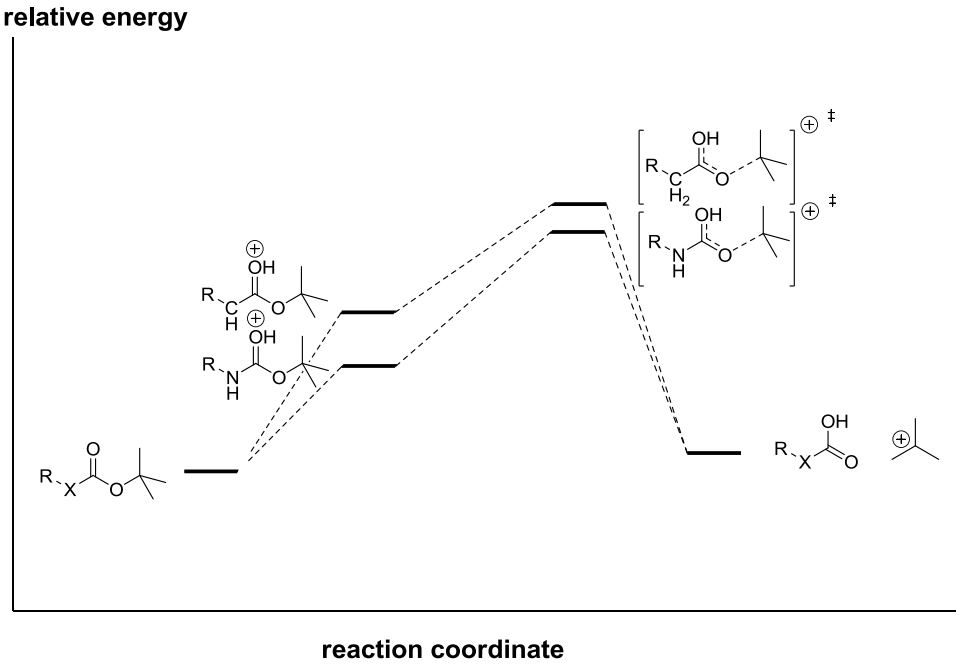


Figure 48 Energy diagram, protonation and breaking of the O-C bond in ^{t}Bu amide and a ^{t}Bu ester.

Methods using 4 M HCl in 1,4-dioxane, and *para*-toluenesulfonic acid in ethanol were unsuccessful - both the Boc group and the tertiary butyl ester group were removed. Finally methanesulfonic acid was used[132] - the reaction was initially carried out in deuterated methanol and followed by ^{1}H NMR spectroscopy. Figure 50 shows the aliphatic region of the ^{1}H NMR spectrum of the starting material. The Boc and tertiary butyl groups, as well as the peaks for the protons at the stereocentres of tryptophan and lysine can be clearly observed. Methanesulfonic acid was added and a ^{1}H NMR spectrum was taken after 48 hours (Figure 51). The Boc group peak has disappeared while the tertiary butyl ester group remains. In addition the proton at the stereocentre of tryptophan has changed and the by-product from the tertiary butyl cation reacting with the solvent methanol is visible. The reaction was heated to 50 ºC overnight and another ^{1}H NMR spectrum was taken (Figure 52). The ^{1}H NMR spectrum shows the Boc group and the tertiary butyl ester groups have been removed. This study reveals that although the deprotection reaction is slow, only the Boc group is removed at room temperature. By heating the reaction the undesired effect of having the tertiary butyl ester group removed occurs.

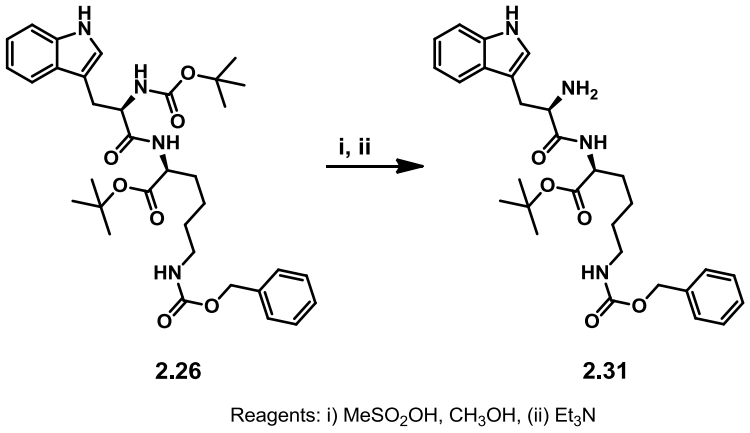


Figure 49 Synthesis of **2.31** from **2.26**

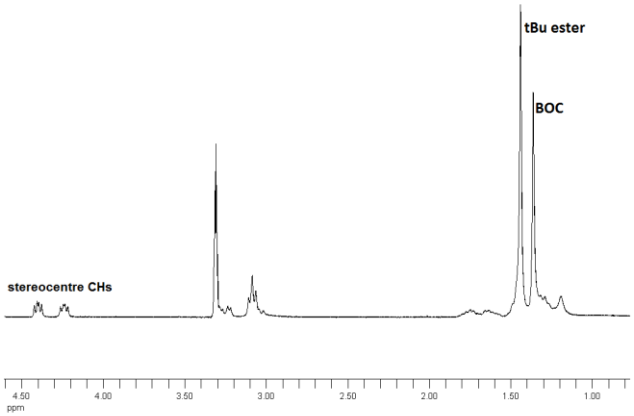


Figure 50 Aliphatic region of ^{1}H NMR spectrum of (**1.26**) taken before addition of $MeSO_2OH$ (t = 0), Boc and ^{t}Bu ester peaks are labelled as are the two peaks for the protons at stereocentres

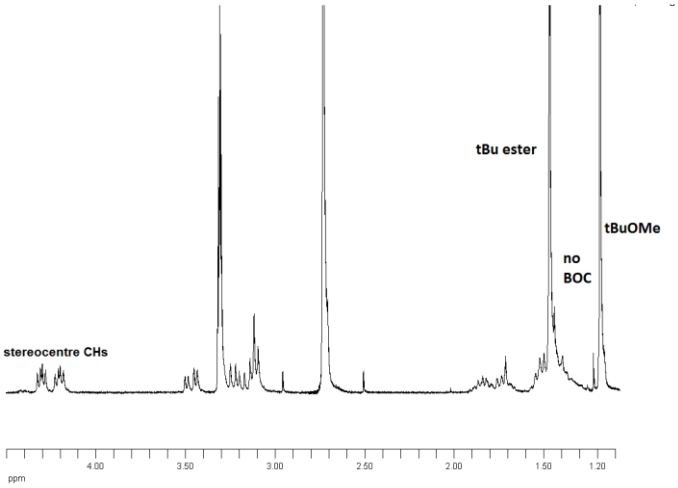


Figure 51 Aliphatic region of ^{1}H NMR spectrum taken after addition of $MeSO_2OH$ (t = 48 hours), Boc group peak has disappeared, ^{t}Bu ester peak is present, ^{t}BuOMe is the by-product

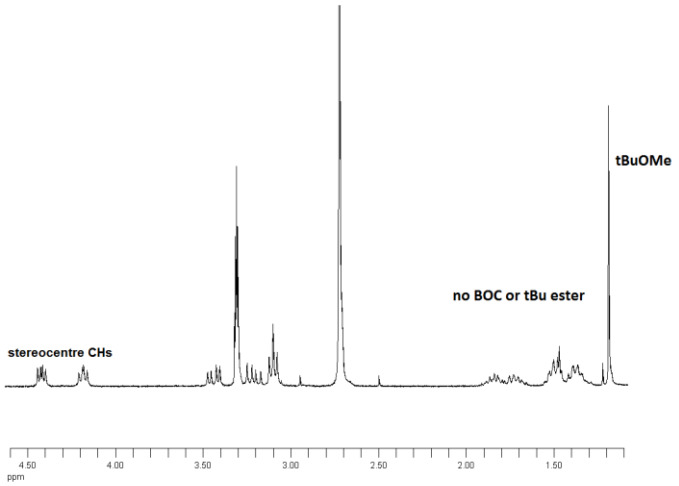
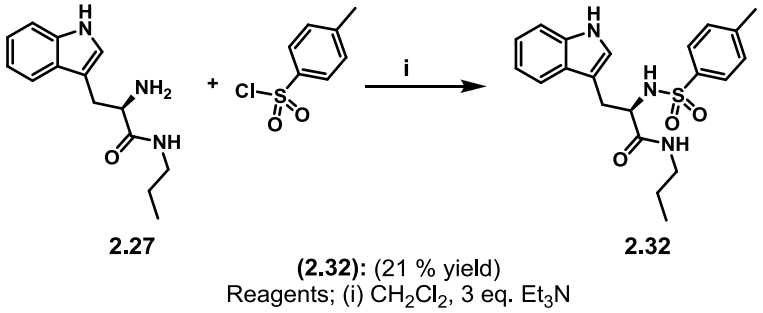


Figure 52 Aliphatic region of ^{1}H NMR spectrum after being heated to 50 ºC (t = 48 hours), both Boc and ^{t}Bu ester groups have gone, peak visible is the by-product ^{t}BuOMe

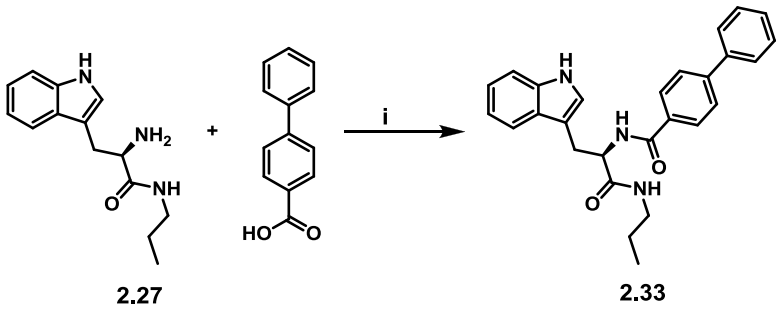
2.2.7 Lysine-Tryptophan-Phenylalanine Mimics

Sulfonamide **2.32** was synthesised by coupling 4-toluenesulfonyl chloride to amine **2.27** (Scheme 7).



Scheme 7 Synthesis of compound **2.32**

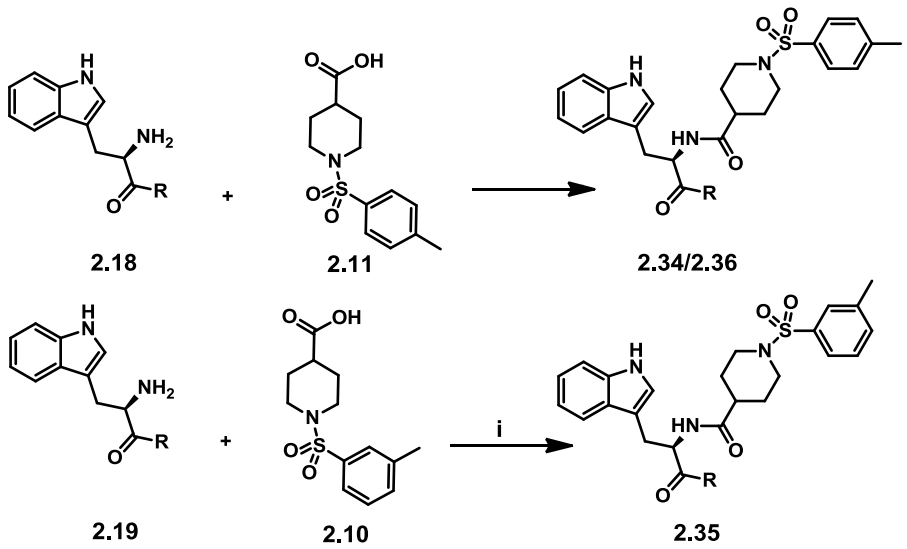
Compound **2.33** was synthesised from amine **2.27** using HATU as the coupling reagent with triethylamine as a base (Scheme 8).



(2.33): (63 % yield)
Reagents; (i) CH_2Cl_2, HATU, 3 eq. Et_3N

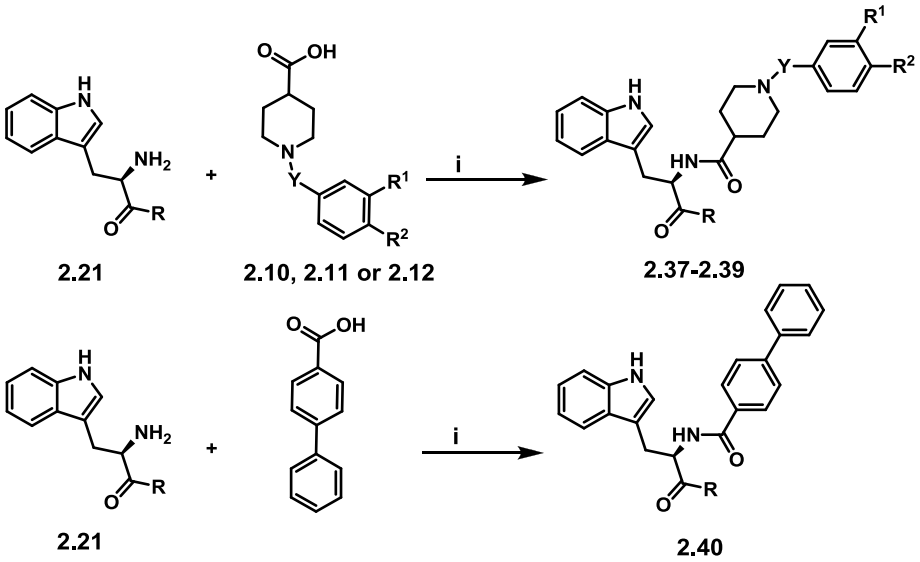
Scheme 8 Synthesis of compound **2.33**

Compound **2.34** was synthesised with amine **2.18** as the lysine motif, compounds **2.35** and **2.36** (Scheme 9) were synthesised using amine **2.19** whilst compounds **2.37**-**2.40** were synthesised using amine **2.21** (Scheme 10).



(2.34): 47 % yield, **(2.35):** 49 % yield,
(2.36): 25 % yield
Reagents; (i) CH_2Cl_2, HATU, 3 eq. Et_3N

Scheme 9 Synthesis of compounds **2.34-2.36**

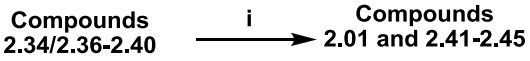


(2.37): R^1=H, R^2=Me, Y=SO_2 (51 % yield), **(2.38):** R^1=Me, R^2=H, Y=SO_2 (63 % yield),
(2.39): R^1=H, R^2=H, Y=CO (55 % yield), **(2.40):** (12 % yield)
Reagents; (i) CH_2Cl_2, HATU, 3 eq. Et_3N

Scheme 10 Synthesis of compounds **2.37**-**2.40**

2.2.8 Removal of the Cbz Groups

The final stage was to remove the Cbz group from carbamates **2.34-2.40** to release the amine functionality of the lysine mimics (Scheme 11). This was done by a hydrogenolysis reaction.[132] In total six SSTR2 ligands were synthesised in this way, with four containing the tertiary butyl ester motif (Figure 53, **2.01** and **2.41**-**2.45**).



Reagents; (i) CH_3OH/THF, Pd/C, H_2, Y

Scheme 11 Synthesis of compounds **2.01** and **2.41**-**2.45**

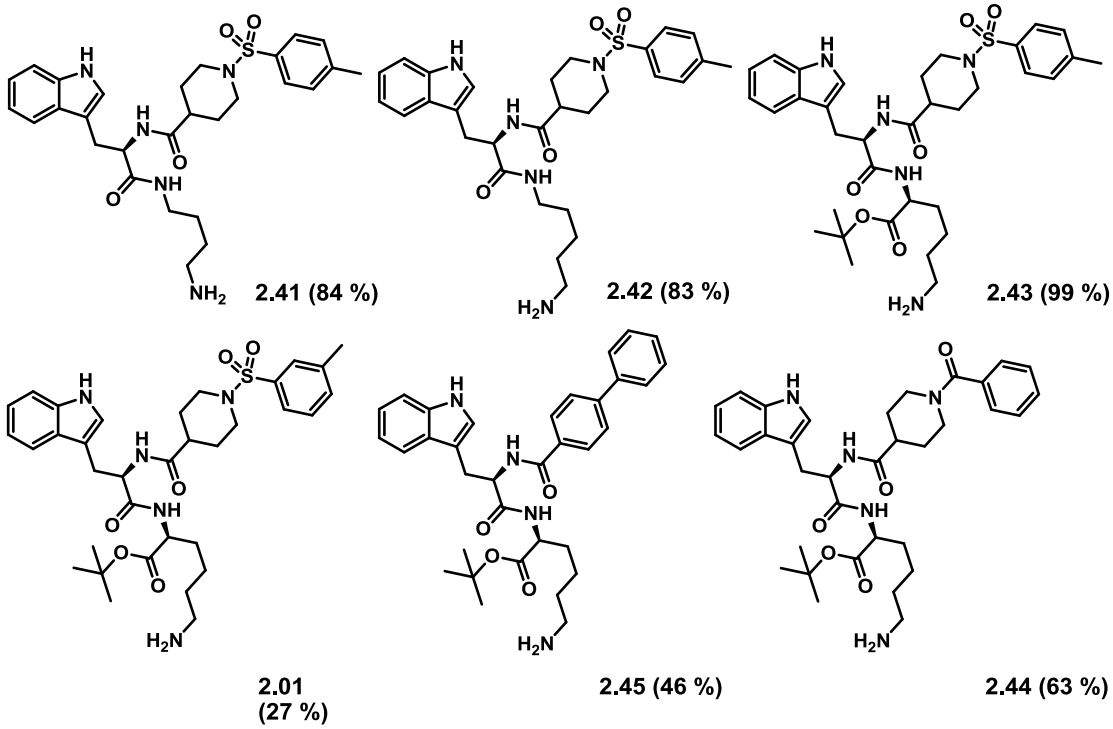
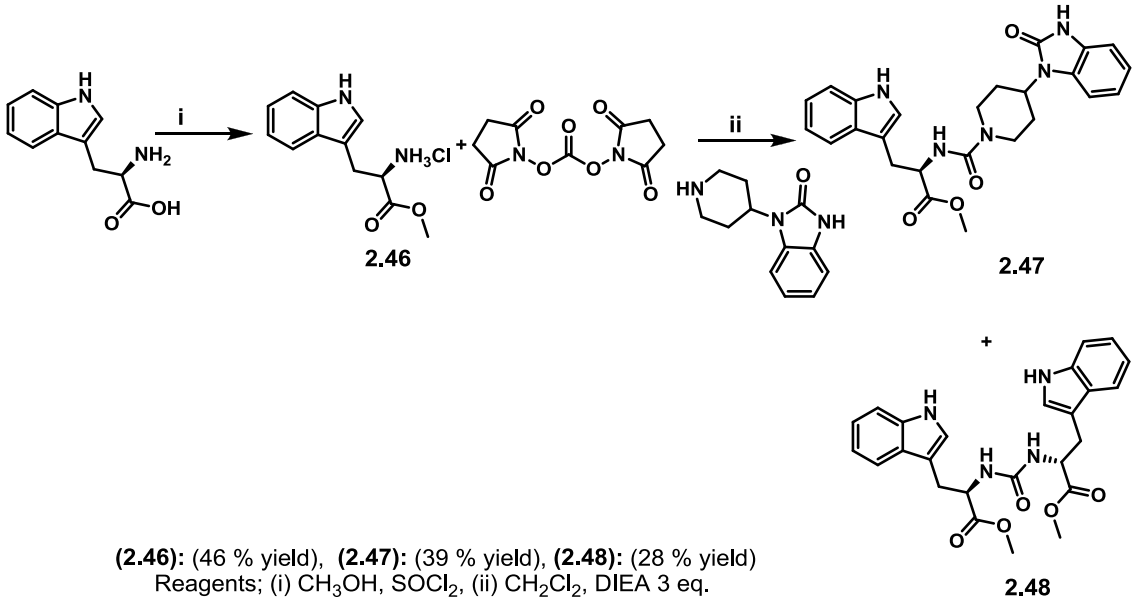


Figure 53 Compounds **2.01** and **2.41**-**2.45** (isolated yields shown in brackets)

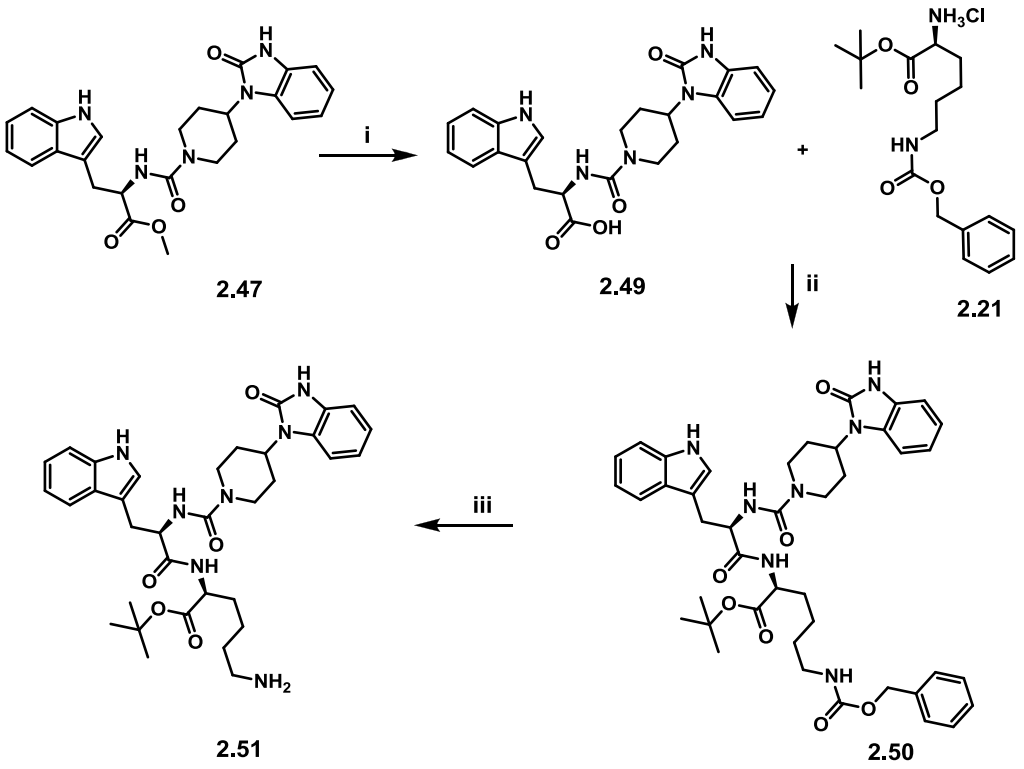
2.2.10 Another KWF mimic

One final urea-containing SSTR2 ligand was synthesised using a different method.[188] Disuccinimidyl carbonate was used as a coupling reagent. This provides a carbonyl for the commercially available 4-(2-keto-1-benzimidazolinyl)-piperidine to react with to form a urea link between the tryptophan and the phenylalanine motif (Scheme 12). D-Tryptophan methyl ester **2.46** was synthesised from D-tryptophan using thionyl chloride in methanol, and the free amine was reacted with disuccinimidyl carbonate and 4-(2-keto-1-benzimidazolinyl)piperidine. Two products were formed from this reaction - the desired product compound **2.47**, and a symmetrical tryptophan methyl ester-containing urea **2.48**. The two products were separated by flash column chromatography.



Scheme 12 Synthesis of compounds **2.46** and **2.47** and unwanted product **2.48**

Methyl ester **2.47** was hydrolysed with lithium hydroxide in tetrahydrofuran and D_2O to give acid **2.49** (Scheme 13). D_2O was used as a solvent so that if epimerisation of the stereocentre occurred it would be highlighted by a reduction in the integral of the stereocentre bound hydrogen in the ^1H NMR spectrum. No reduction in the integral was observed, indicating epimerisation did not occur. Acid **2.49** was coupled to amine **2.21** to give carbamate **2.50**. The Cbz group was removed as for the previous compounds by hydrogenolysis to give the final SSTR2 like ligand compound **2.51**.



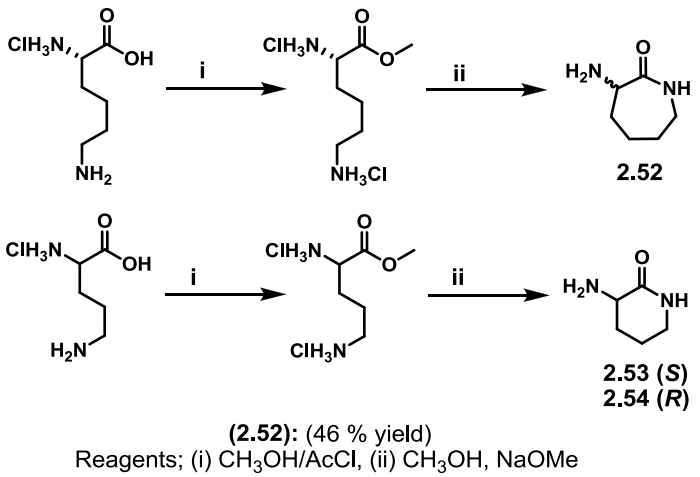
(2.49): (93 % yield), **(2.50):** (25 % yield), **(2.51):** (55 % yield)
Reagents; (i) THF/D_2O/EtOH, LiOH, (ii) CH_2Cl_2,
HATU, 3 eq. Et_3N, (iii) CH_3OH, Pd/C, H_2

Scheme 13 Synthesis of compounds **2.49**-**2.51**

2.3 Lactam Containing Molecules

2.3.1 Glutamine Mimics - Lactam Synthesis

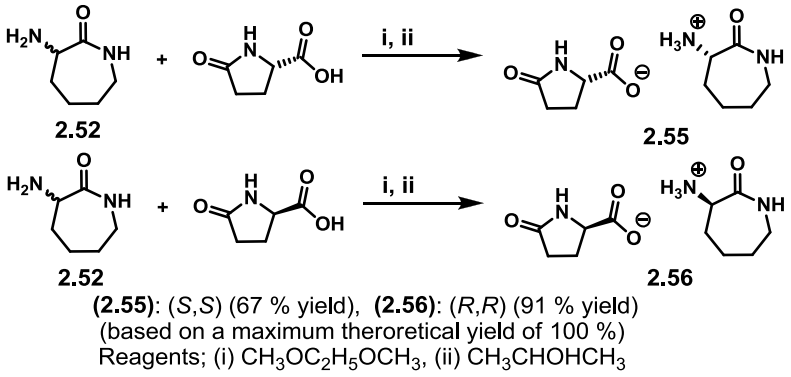
6-Membered and 7-membered lactams were synthesised by cyclisation of L-ornithine and L-lysine (Scheme 14, **2.52-2.54**).[189] The 7-membered lactam **2.52** was isolated as a colourless oil which formed white crystals when left to stand, the 6-membered lactams **2.53** and **2.54** were not isolated.



(2.52): (46 % yield)
Reagents; (i) $CH_3OH/AcCl$, (ii) CH_3OH, NaOMe

Scheme 14 Synthesis of compounds **2.52**-**2.54**

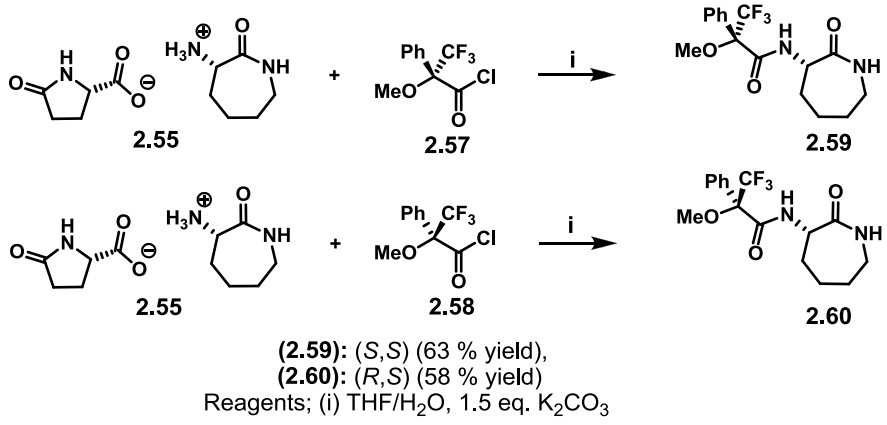
After addition of the base, the 6-membered lactam is formed at a temperature under 0 °C. In these mild conditions enolisation of the amino ester or amino lactam product does not occur and so the stereochemistry of the ester is maintained in the product lactam. The rate of cyclisation to give 7-membered rings is lower than that for 6-membered rings therefore the 7-membered lactam only forms at a reasonable rate when the reaction is heated under reflux conditions. These harsher conditions results in racemisation of the product. The racemic 7-membered ring was resolved using enantiomerically pure pyroglutamic acid. The free amine lactam dissolves in dimethoxyethane, and on addition of enantiomerically pure pyroglutamic acid the desired enantiomer of lactam precipitates out as the pyroglutamic acid salt whilst the other enantiomer remains in solution (Scheme 15, **2.55**, **2.56**).[190] Washing with 2-propanol ensured all free lactam was removed.



Scheme 15 Synthesis of compounds **2.55**-**2.56**

2.3.2 Using Mosher's Acid to determine e.e.

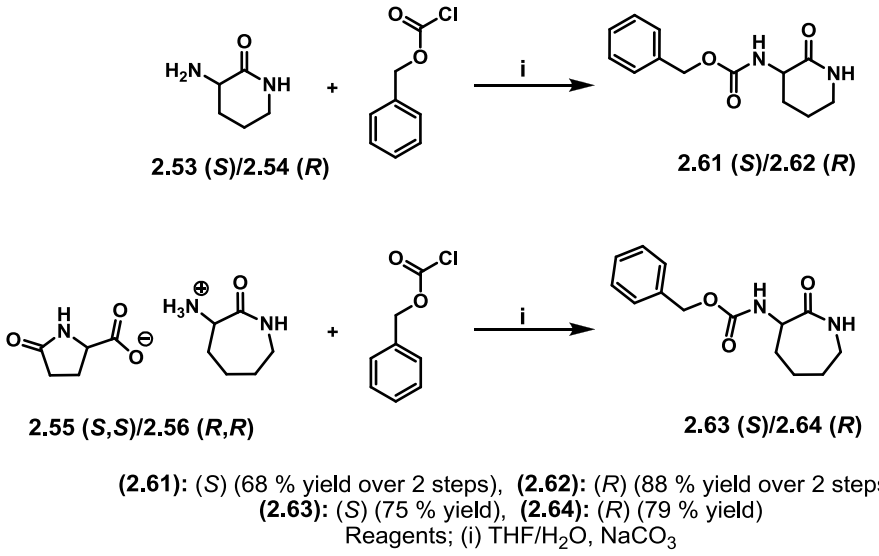
To determine the enantiomeric excess of the resolved lactam it was coupled to an enantiomerically pure compound, α-methoxy-α-trifluoromethylphenylacetic acid (MTPA), known as Mosher's acid, to make diastereoisomers.[191] This was done *via* the acid chlorides compounds **2.57** and **2.58**. The enantiomeric excess of lactam **2.55**, calculated from the diastereomeric compounds **2.59** and **2.60**, was 95 % (from compound **2.59**) and 98 % (from compound **2.60**). The compounds were washed in hot propan-2-ol before further optical testing.



Scheme 16 Synthesis of compounds **2.59** and **2.60**

2.3.3 Using Chiral HPLC to Determine the e.e.

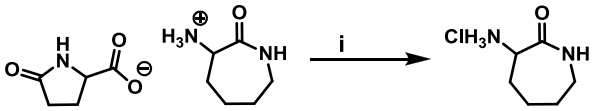
To ensure the optical purity of all the lactams a faster technique was required. Chiral high-performance liquid chromatography (HPLC) proved to be an appropriate, successful and faster method. Sufficient separation of the lactams required the amine functionality of the lactams to be Cbz protected[192] - this was achieved by reacting the lactams with benzylchloroformate (Scheme 17, **2.61**-**2.64**).



(2.61): (*S*) (68 % yield over 2 steps), **(2.62):** (*R*) (88 % yield over 2 steps),
(2.63): (*S*) (75 % yield), **(2.64):** (*R*) (79 % yield)
Reagents; (i) THF/H_2O, $NaCO_3$

Scheme 17 Synthesis of compounds **2.61**-**2.64**

Pre-resolution, the lactam ring was shown to be racemic following Cbz protection and chiral HPLC. The resolved lactams of each enantiomer, after a final wash with hot propan-2-ol, were shown to have an enantiomeric excess of ≥ 99 %. The hydrogen chloride salts of the 7-membered lactams were generated in an ion exchange reaction. Dissolving acetyl chloride in ethanol creates HCl *in situ* and adding the pyroglutamic acid lactam salt results in the hydrogen chloride salt of the lactam precipitating from solution (Scheme 18, **2.65**, **2.66**).



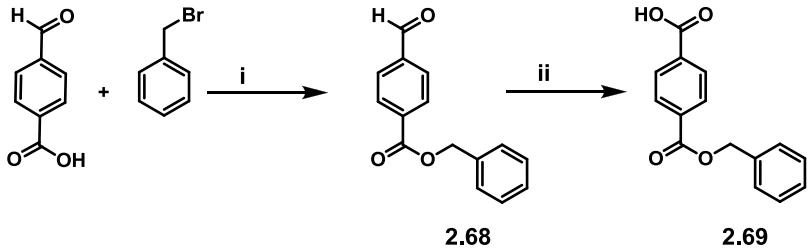
2.55 (*S*, *S*)
2.56 (*R*, *R*)

2.65 (*S*)
2.66 (*R*)

(2.65): (*S*), (93 % yield), **(2.66):** (*R*), (99 % yield)
Reagents; (i) C_2H_5OH, AcCl

Scheme 18 Synthesis of compounds **2.65** and **2.66**

4-Carboxybenzaldehyde was benzylated (Scheme 19) with a yield of 80 % to give aldehyde **2.68**. The reaction was performed in DMF with sodium hydride as the base and benzyl bromide as the electrophile.[193] *N*, *N*, *N′*, *N′*-Tetramethylethylenediamine (TMEDA) or 1,4-diazabicyclo[2.2.2]octane (DABCO) were used to wash out excess benzyl bromide. The amine is monoalkylated by benzyl bromide and the product can be washed into *p*H 2 buffer. Residual DMF can be removed by washing with 0.1 M aqueous HCl. Sodium chlorite and sulfamic acid are used to oxidise the aldehyde.[193] Sulfamic acid is a scavenger which reacts with the hypochlorous acid by-product to prevent this unwanted reaction.[194]



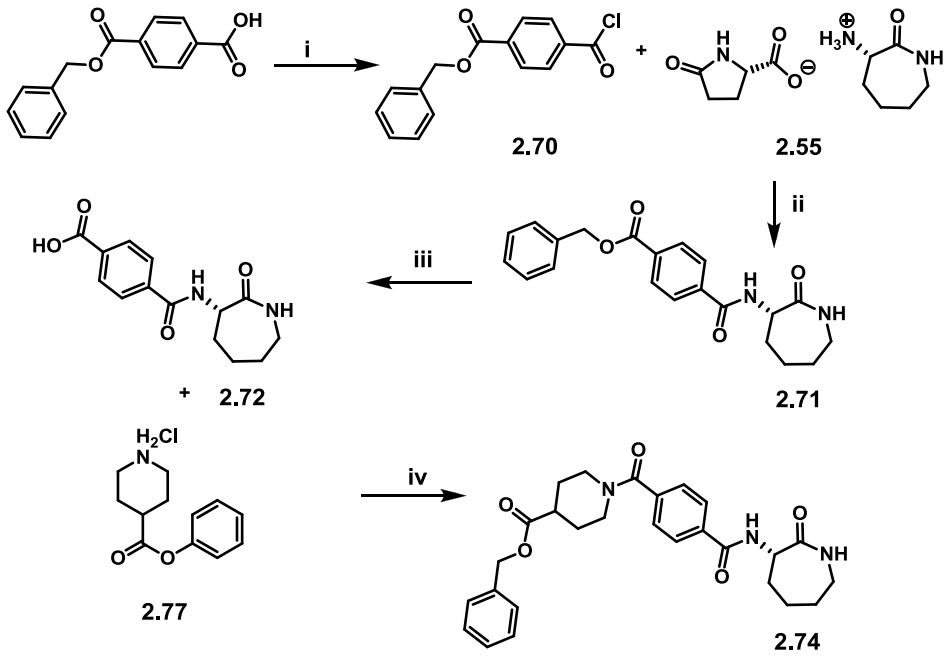
2.68 **2.69**

(2.68): (80 % yield), **(2.69):** (22 % yield)
Reagents; (i) DMF, NaH, (ii) CH_3CN, $NaClO_2/NH_2SO_2OH$

Scheme 19 Synthesis of compounds **2.68** and **2.69**

Compound **2.69** was used to synthesise phenylalanine-glutamine mimics **2.71-2.74** (Figure 54). Acid **2.69** was converted to its acid chloride with oxalyl chloride and catalytic DMF (Scheme 20). Acid chloride **2.70** was reacted with the 7-membered

lactam pyroglutamic acid salt **2.55** and the 6-membered lactam **2.53** in a Schotten-Baumann reaction[185] to give amides **2.71** and **2.73**. The benzyl group in **2.71** was removed by hydrogenolysis to give acid **2.72**. Finally, acid **2.72** was coupled to isonipecotic acid benzyl ester hydrogen chloride salt **2.77** (*vide infra*) to give tertiary amide **2.74**. Because of the low yields involved in synthesising terephthalic acid monobenzylester **2.69** (18 % over two steps), another phenylalanine mimic was chosen in order to allow the efficient synthesis of a large number of compounds including this motif.



(2.71): (10 % yield), **(2.72):** (95 % yield), **(2.74):** (45 % yield)
Reagents; (i) $CH_2Cl_2/(COCl)_2$, DMF, (ii) CH_2Cl_2/H_2O, 3 eq. Et_3N, (iii) MeOH, Pd/C, H_2, (iv) CH_2Cl_2, HATU, 3 eq. Et_3N

Scheme 20 Synthesis of compounds **2.70**-**2.74**

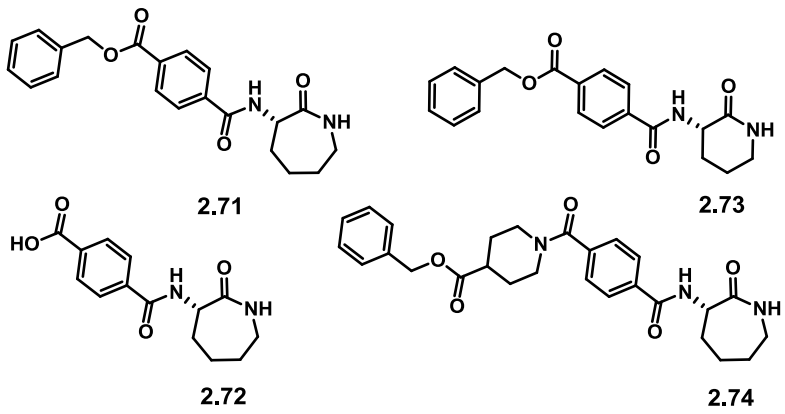
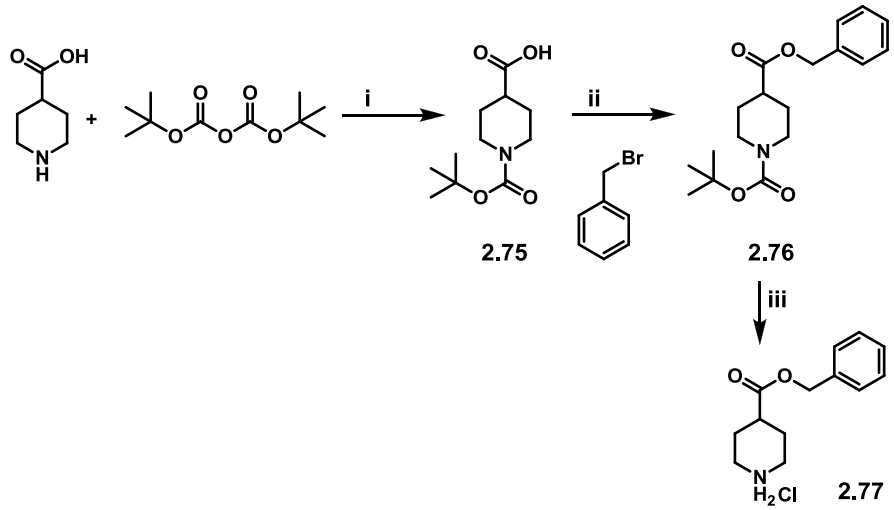


Figure 54 Compounds **2.71**-**2.74**

2.3.5 Sulfonamide Phenylalanine Mimics

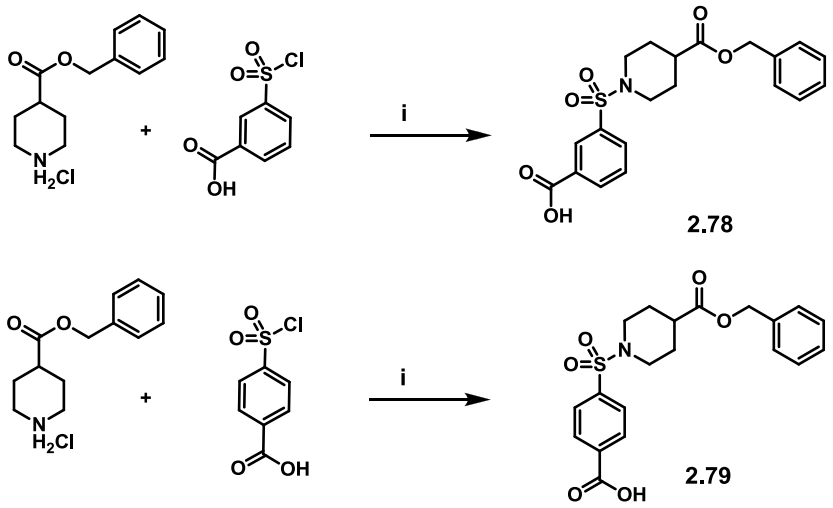
Isonipecotic acid was protected with di-*tert*-butyldicarbonate (Scheme 21, **2.75**). The carboxylic acid **2.75** was then benzyl-protected using benzyl bromide to give compound **2.76**. The Boc group was removed to give compound **2.77**.



Scheme 21 Synthesis of compounds **2.75**-**2.77**

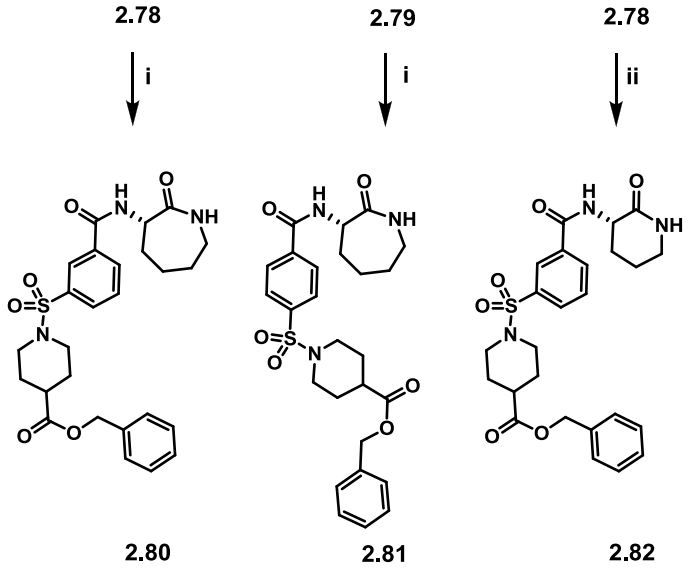
Amine **2.77** was coupled to both 3-(chlorosulfonyl)benzoic acid and 4-(chlorosulfonyl)benzoic acid under Schotten-Baumann conditions[185] (Scheme 22). The sulfonamide products **2.78** and **2.79** were coupled to the lactams either using

HATU as a coupling reagent, or by making an acid chloride and coupled under Schotten-Baumann conditions[185] (Scheme 23, **2.80**-**2.82**).



(2.78): (43 % yield), **(2.79):** (88 % yield)
Reagents; (i) CH_2Cl_2, 3 eq. Et_3N

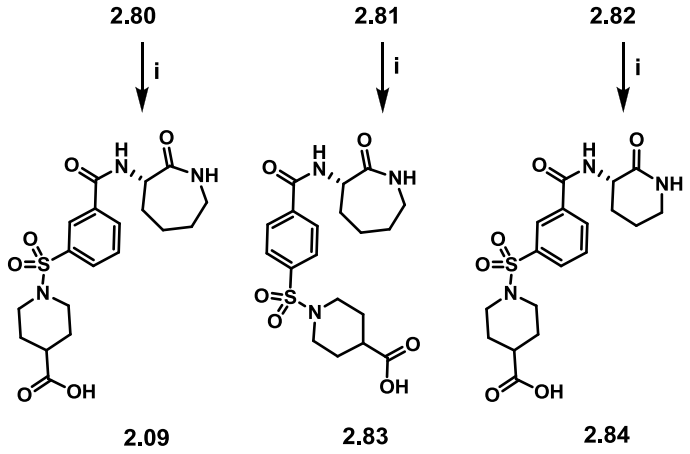
Scheme 22 Synthesis of compounds **2.78**-**2.79**



(2.80): (75 % yield), **(2.81):** (57 % yield),
(2.82): (50 % yield)
Reagents; (i) CH_2Cl_2, HATU, 3 eq. Et_3N, **(2.65)**,
(ii) CH_2Cl_2, H_2O, 3.9 eq. K_2CO_3, **(2.53)**

Scheme 23 Synthesis of compounds **2.80**-**2.82**

The benzyl esters of compounds **2.80-2.82** were removed by hydrogenolysis to give compounds **2.09** and **2.83-2.84** (Scheme 24).



Scheme 24 Synthesis of compounds **2.09** and **2.83-2.84**

The FQ mimics (Figure 55, **2.09** and **2.83-2.84**) were coupled to the KW amines tryptamine and compounds **2.27-2.31**, using HATU as a coupling reagent.

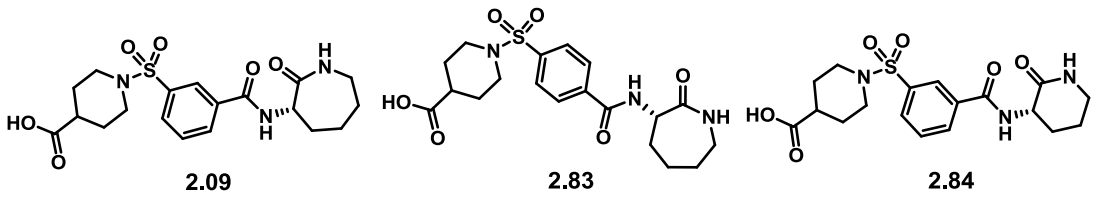


Figure 55 Compounds **2.09** and **2.83-2.84**

Compounds **2.07**, **2.08** and **2.85-2.87** were synthesised using tryptamine or amine **2.27** using HATU as a coupling reagent (Figure 56).

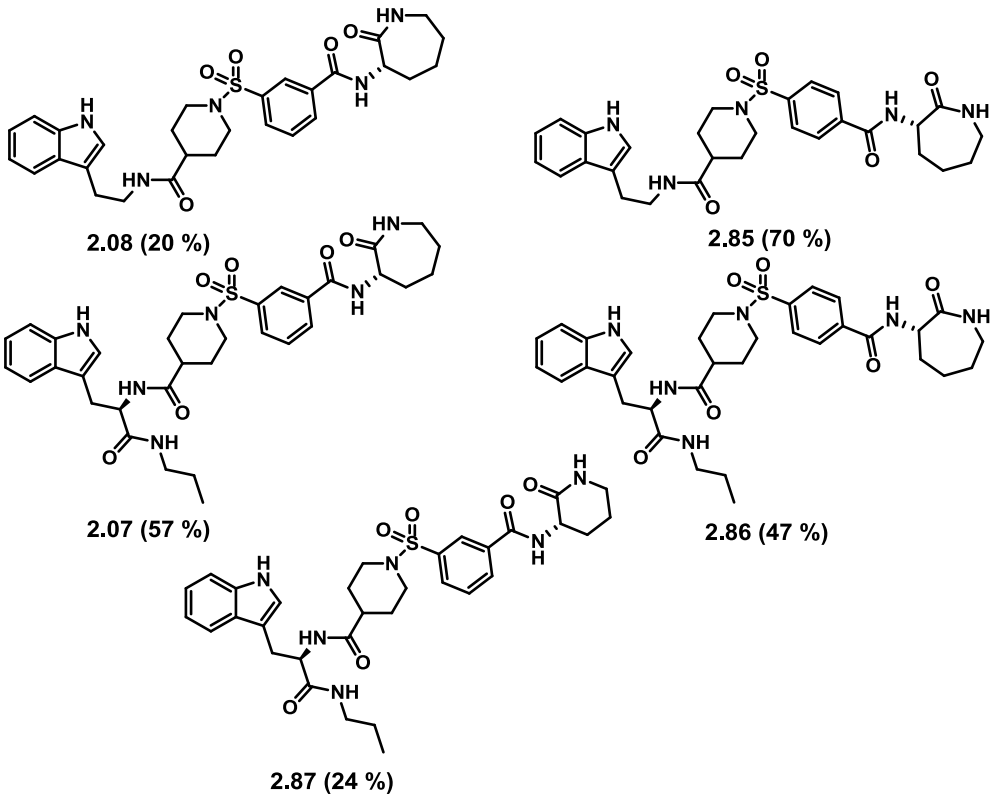


Figure 56 Compounds **2.07**-**2.08** and **2.85**-**2.87 (**isolated yields are shown in brackets)

Compounds **2.88**-**2.92** were synthesised using amines **2.28**-**2.31** using HATU as a coupling reagent (Figure 57,). Finally the Cbz groups were removed through hydrogenolysis reactions to give the largest hybrid compounds, the KWFQ mimics compounds **2.93**-**2.96** (Figure 58).

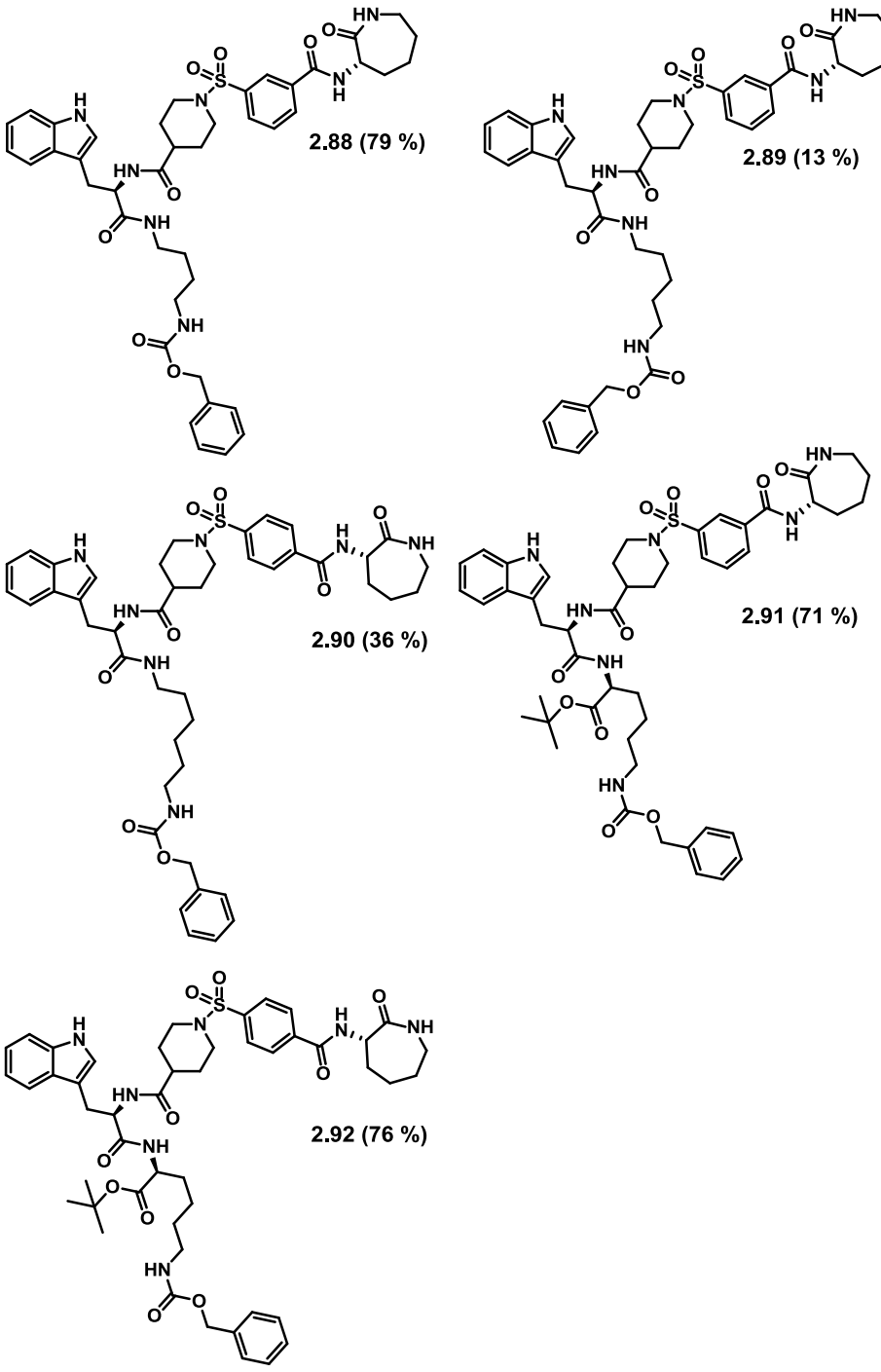


Figure 57 Compounds **2.88**-**2.92** (isolated yields are shown in brackets)

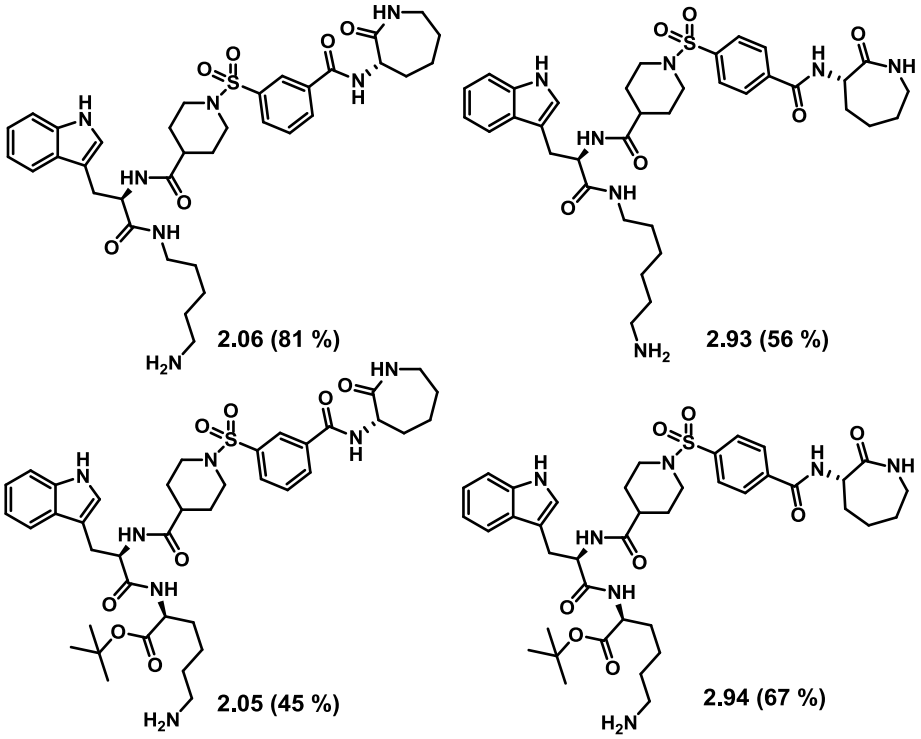


Figure 58 Compounds **2.05-2.06** and **2.93-2.94** (isolated yields are shown in brackets)

2.4 Biological Testing

The compounds were subjected to two different biological assays, an SSTR2 binding assay and a leukocyte migration assay to determine BSCI activity.

2.4.1 SSTR2 Binding

SSTR2 binding data was obtained by Tilly Sharp at Total Scientific (Babraham Research Campus, Cambridge) using a technique called fluorescence polarisation (FP). This technique gives a measure of the amount each compound binds to SSTR2 in comparison to fluorescently labelled somatostatin, the native ligand for SSTR2. FP is based on the theory initially described by Perrin in 1926 that small molecules, when excited by plane polarised light, can emit largely depolarised light as a result of rapid molecular tumbling in solution.[195] Small molecules that are bound to a receptor have an effective increase in molecular volume and tumble much slower in solution. Therefore, when excited by plane polarised light, the emitted light remains

polarised to a greater extent.[196] The FP of somatostatin bound to SSTR2 can be compared to the FP of somatostatin that has been displaced by the test compound. If the compound displaces somatostatin, the non-bound somatostatin will cause the emission of depolarised light. Conversely, if the compound does not displace somatostatin, the emitted light will remain polarised (Figure 59). The FP assay was run following a standard protocol. Emitted light is measured in the vertical and horizontal planes and the polarisation is calculated.

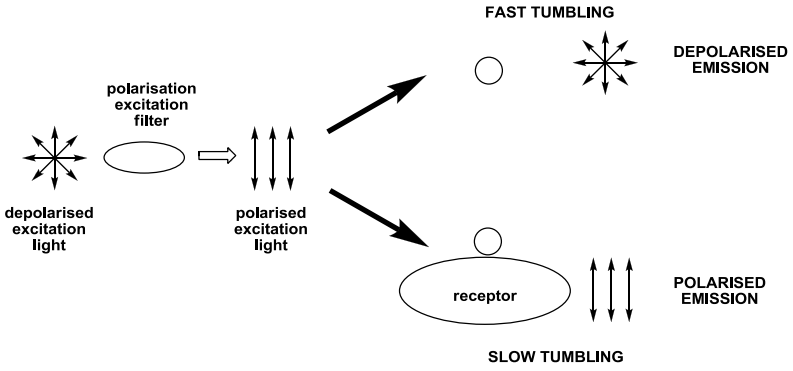


Figure 59 Fast tumbling small molecules depolarise light, slow tumbling large molecules allows light to remain polarised

The WF, KWF, KWFQ, WFQ and FQ mimics were tested for their SSTR2 binding activity at a concentration of 1 nM, the results are shown in Table 1.

The data is given as percentage inhibition of SS-14 FITC from SSTR2. The WF, WFQ and FQ compounds showed no significant binding. These compounds are lacking the lysine motif which is known to promote strong binding in these types of ligands.[197] The KWF compounds (**2.01**, **2.45** and **2.51**), based on the structure of current SSTR2 ligands, all show high activity as expected. The KWFQ compounds (**2.05** and **2.94**), based on the structure of current SSTR2 ligands with the addition of the lactam, showed equally high activity. The key findings of these results are that

the full lysine motif is required for SSTR2 binding and that the addition of the lactam is not significantly detrimental to binding.

Family	Compound	% Inhibition	Family	Compound	% Inhibition
WF	**2.04**	-2	WFQ	**2.07**	-17
	2.14	-7		**2.08**	-9
	2.15	0		**2.85**	-25
	2.32	-3		**2.86**	-6
	2.33	-18		**2.87**	-18
KWF	**2.01**	105	FQ	**2.09**	5
	2.45	96		**2.71**	-15
	2.51	97		**2.72**	6
KWFQ	**2.05**	98		**2.73**	3
	2.94	75		**2.80**	17
				2.81	8

Table 1% inhibition of SS-14 FITC at SSTR2, compounds a 1 nM, values are given with an estimate error ± 20 % (data from Tilly Sharp at Total Scientific)

The errors associated with measurement are about 20 % in this assay. The errors associated with the effective concentration of the test compound are due to distribution and solubility. Their significance depends on the position in the binding curve. The binding curve is sigmoidal, meaning the errors close to 0 and 100 % are less significant than the errors at 50 %. The majority of data from these assays is in the former regions.

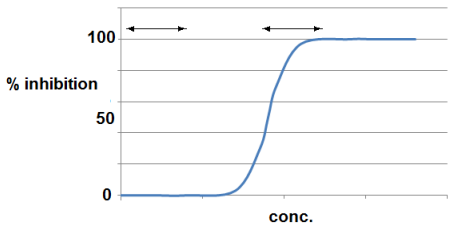


Figure 60 % inhibition, minimal at 0 and 100 %, greatest at 50 % inhibition

The half maximal inhibitory concentration (IC_{50}) and binding affinity (K_i) values were calculated for the five active compounds. The percentage inhibition of the compounds was measured at different concentrations and plotted against the log of these concentrations. A sigmoidal curve (Equation 1) was fitted to the data to determine the IC_{50} value and the hill slope. The hill slope is the number of substrates which bind each receptor and was approximately one for these compounds.

$$y = \frac{100}{1+10^{((\log IC_{50}-x)*HillSlope)}}$$

Equation 1

The K_i was calculated using the Cheng-Prusoff equation,[198] Equation 2 where S is the concentration of test compound and K_m is the concentration of SS-14 FITC at which binding is half maximal.

$$K_i = \frac{IC_{50}}{1+\frac{[S]}{K_m}}$$

Equation 2 Cheng-Prusoff equation[198]

The K_m of SS-14 FITC was determined by titrating it against unlabelled somatostatin, the K_m of which was taken from the literature as 0.23 nM.[199] This gave

a K_i value of 0.384 nM. This value of 0.384 nM was used as the K_m to calculate the K_i values of the test compounds (Table 2, Figure 61).

Family	Compound	IC_{50} (nM)	Hill slope	K_i (nM)
KWF	**2.01**	4.21 ± 1.24	1.15 ± 0.251	1.17
	2.45	32.2 ± 1.19	1.32 ± 0.251	8.93
	2.51	0.870 ± 1.18	1.89 ± 0.548	0.241
KWFQ	**2.05**	55.0 ± 1.15	1.36 ± 0.222	15.3
	2.94	110 ± 1.09	1.19 ± 0.115	30.5

Table 2 SSTR2 binding IC_{50} and K_i values

The data was than fitted to a slightly modified equation (Equation 3) taking into account that some of the maximal inhibition levels are above 100 % (Table 3, Figure 62).

$$y = \frac{\text{max.inhibition}}{1+10^{((\log IC_{50} - x)*\text{HillSlope})}}$$

Equation 3

Family	Compound	IC_{50} (nM)	Hill slope	Max. Inhib.	K_i (nM)
KWF	**2.01**	6.47 ± 1.20	0.965 ± 0.128	119 ± 4.57	1.80
	2.45	66.9 ± 1.19	0.858 ± 0.082	133 ± 6.61	18.6
	2.51	1.08 ± 1.08	1.45 ± 0.144	115 ± 1.81	0.300
KWFQ	**2.05**	93.4 ± 1.24	0.965 ± 0.18	125 ± 8.64	25.9
	2.94	140 ± 1.26	1.04 ± 0.151	110 ± 8.96	38.8

Table 3 SSTR2 binding IC_{50} and K_i values using Equation 3

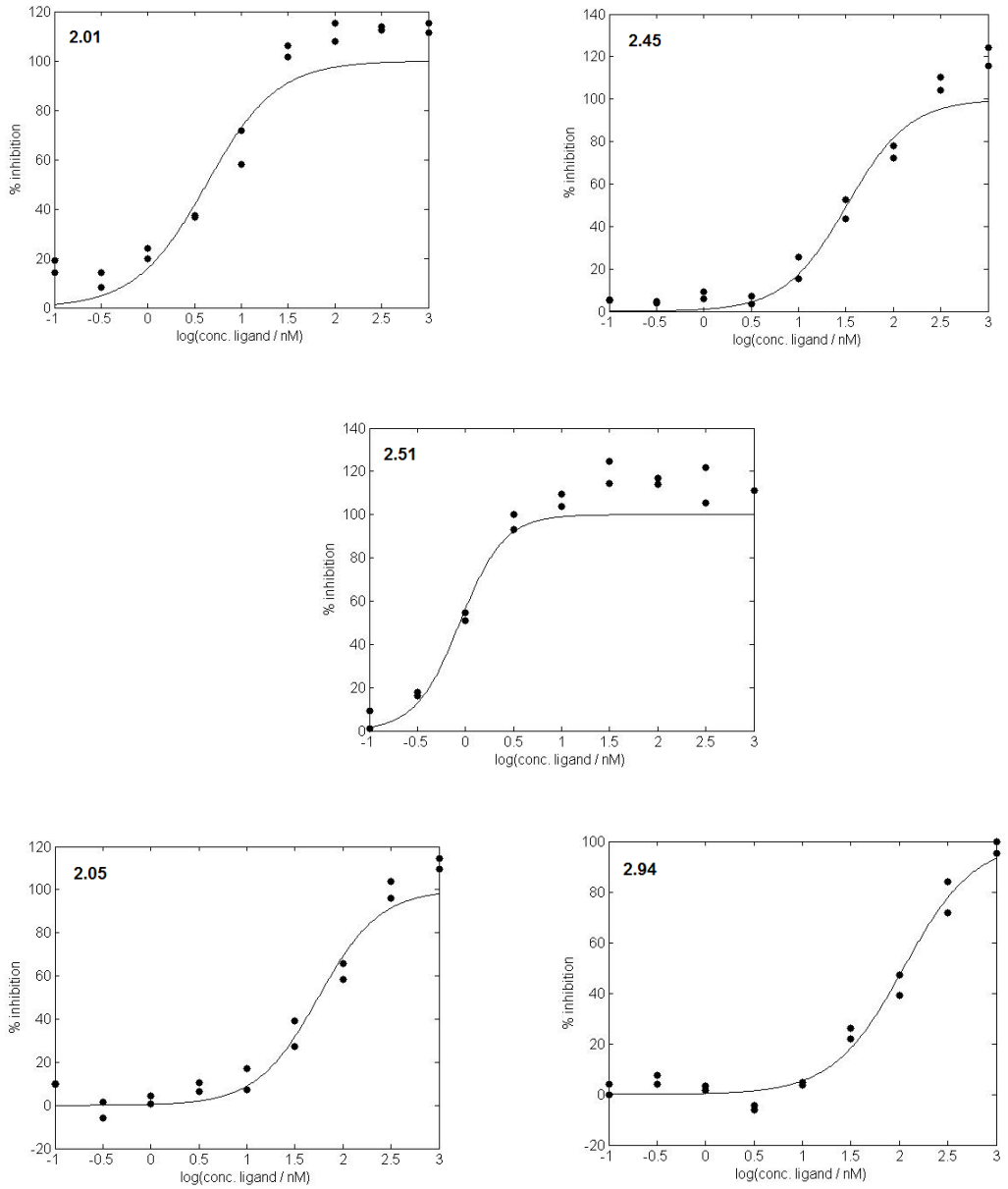


Figure 61 Titration curves, x-axis is log of the concentration of test compound, y-axis is % inhibition of SS-14 FITC

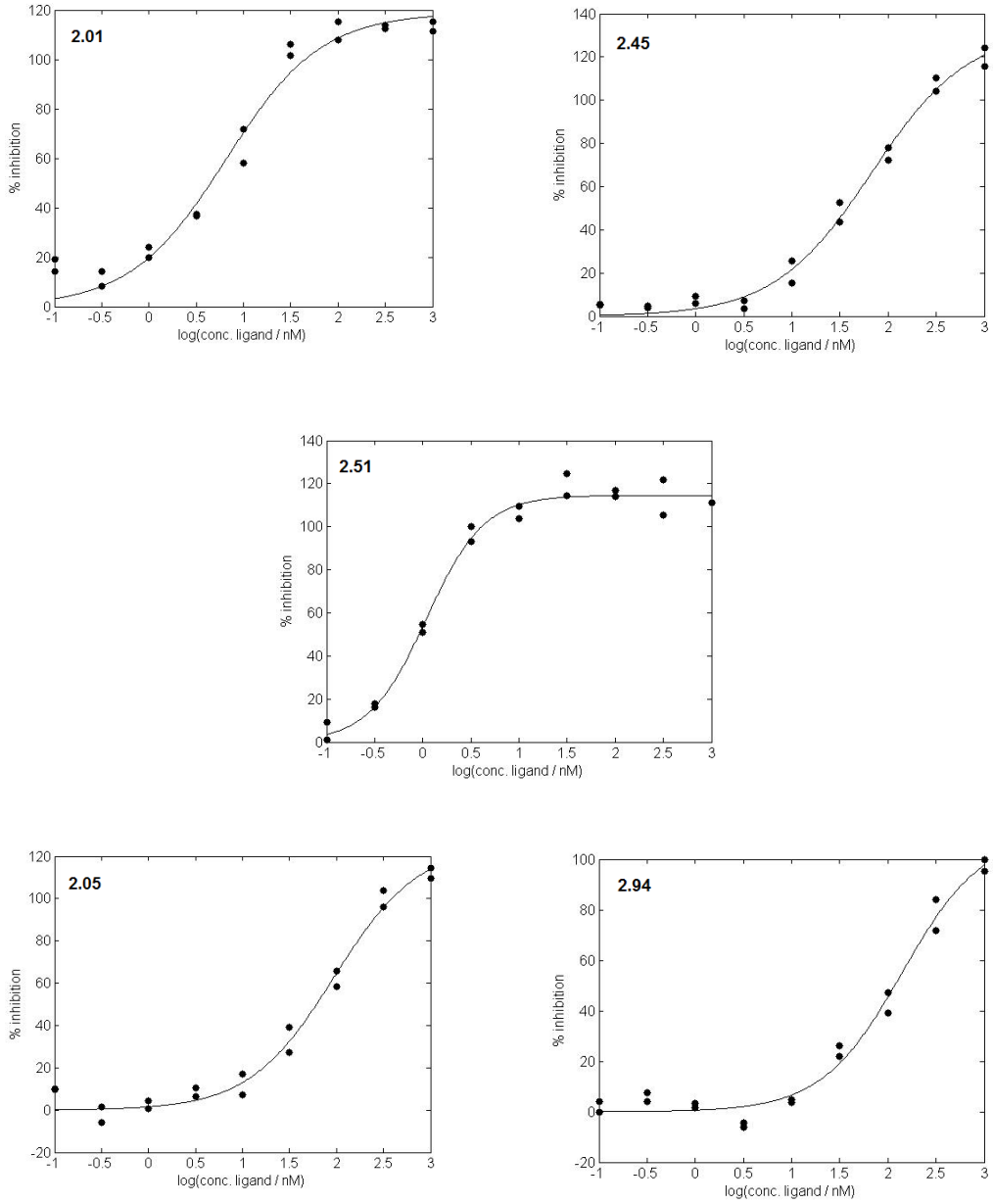


Figure 62 Titration curves using Equation 3, x-axis is log of the concentration of the test compound, y-axis % inhibition of SS-14 FITC

These curves take into account the outlying values of the previous curves and consequently the fit is better, the errors are reasonably constant however. This data shows the three KWF compounds are in general more potent than the KWFQ compounds with compound **2.51** being the most potent.

2.4.2 Leukocyte Migration Assay

Leukocyte migration data was determined by Dr Jill Reckless of The Department of Medicine at The University of Cambridge, using a multi-well filter migration assay system protocol (Figure 63).[200] The migrated cells were quantified using the dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). In the presence of reductases, the tetrazolium ion was reduced to a formazan (Scheme 25). The concentration of reductases was related to the number of living cells, so measuring the amount of formazan is a way of determining this. Data was given as percentage inhibition of neutrophil migration at a given concentration.

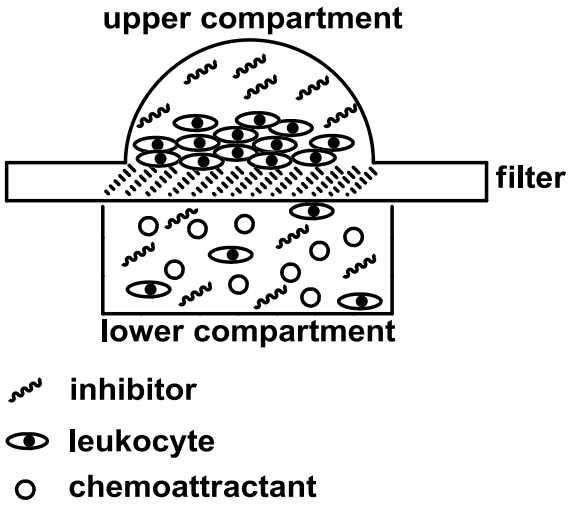
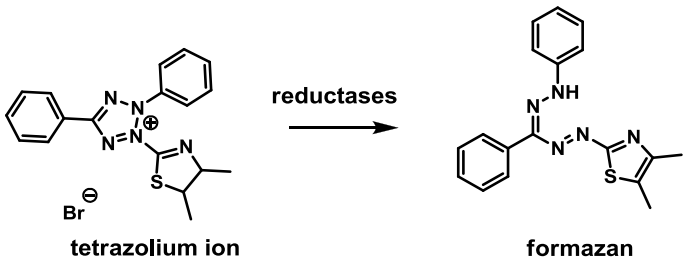


Figure 63 Cross-section of a single migration chamber



Scheme 25 Reduction of a MTT to the corresponding formazan

The five compounds that showed the highest binding activity to SSTR2 - that is the KWF (**2.01**, **2.45** and **2.51**) and KWFQ mimics (**2.05** and **2.94**) - were tested as to their BSCI activity. The ability of the compounds to prevent the chemokine induced migration of the neutrophils was measured at two different concentrations 1 μM and 1 nM and the results are shown in Table 4. Data is given as a percentage inhibition of neutrophil migration. All the compounds show very high inhibition at 1 μM. At 1 nM all the compounds still show high inhibition (over 90 %) apart from lactam **2.94**. Lactam **2.94** inhibits 119 % at 1 μM however at 1 nM this drops down to 7 %. This correlates with the SSTR2 binding, as lactam **2.94** has lower binding activity and a lower IC_{50} value than the other compounds in this group. This study reveals that for these particular ligands high SSTR2 binding correlates to a certain extent with BSCI activity. All the compounds with high SSTR2 binding also have anti-inflammatory activity whether they contain the lactam or not. The position of the lactam is important and decreased SSTR2 binding results in lower BSCI activity.

Family	**Compound**	**% Inhibition ± % error**	
		1 μM	**1 nM**
KWF	**2.01**	123 ± 25	118 ± 19
	2.45	126 ± 21	99 ± 16
	2.51	124 ± 15	94 ± 8
KWFQ	**2.05**	130 ± 15	115 ± 38
	2.94	119 ± 31	7 ± 15

Table 4 % Inhibition of neutrophil migration, errors represent 1 SD (data from Dr Jill Reckless, The University of Cambridge)

2.5 Conclusions

The KWF mimic compounds display high inhibition of somatostatin at SSTR2 which was expected as they were based on existing SSTR2 ligands. Likewise the

KWFQ mimics based on the existing SSTR2 ligands with the addition of the lactam also showed high binding, this shows the addition of the lactam does not have a negative effect on their SSTR2 binding ability. The IC_{50}'s show the KWF mimics are more potent however with compound **2.51** having an approximate 100-fold increase over compound **2.94**. The IC_{50}'s for compounds **2.45**, **2.51** and compounds very close in structure to **2.01** have been reported as 85, 0.26 and between 2.6-2.9 nM.[140] These are close to the values reported herein.

Both the KWF and KWFQ mimics show high inhibition of leukocyte migration at a concentration of 1 μM. At a concentration of 1 nM all of the compounds retain this high activity apart from compound **2.94**. This correlates with the IC_{50} values to a certain extent as compound **2.94** has the highest value. This indicates that in these compounds the ability to displace somatostatin from SSTR2 is linked with the compounds BSCI ability.

It is unexpected however that the KWF compounds act as BSCIs despite lacking the WxQ motif thought essential for BSCI activity. Previous studies have shown while cyclic peptides containing KWIQ act as BSCIs those containing KWI have no BSCI activity and in fact act as inhibitors. BIM58079D, BIM58092D and BIM58078D (compounds **2.95-2.97**) all contain WIQ and have leukocyte migration inhibition potencies of 12, 20 and 80 nM respectively (Figure 64). BIM23454 (compound **2.98**) is a cyclic peptide containing a KWx motif of lysine, (D)-tryptophan and pyridinyl alanine (Pal) (Cpa stands for *p*-chlorophenylalanine and NaI stands for 3-(2-napthyl)alanine). Compound **2.98** has an IC_{50} value of 2.6 nM[139] for the displacement of somatostatin from SSTR2, lacking the WxQ motif it is a potent antagonist against the action of compounds with BSCI activity. This evidence

supports the need the WxQ motif for a compound to have BSCI activity. However the fact that the peptide-like compounds we synthesised did not contain the glutamine residue but still retained BSCI activity suggests that conclusions based on the cyclic BSCIs do not necessarily hold true for the acyclic BSCIs.

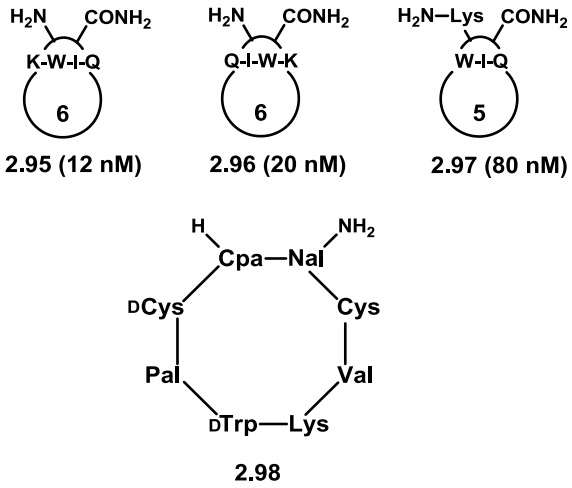


Figure 64 Compounds **2.95-2.98**

Hoyer and colleges have studied the ability of SSTR2 ligands to inhibit forskolin-stimulated adenylate cyclase and thus the production of cyclic adenosine monophosphate (cAMP).[201] The results for compound **2.51** and compounds **1.39** and **2.100** (similar in structure to compound **2.01**) gave inhibition values of 117.9, 117.5 and 119.1 % respectively. These values correlate with the leukocyte migration inhibition data and could indicate cAMP inhibition correlates with leukocyte migration inhibition. To gain more information on this theory the data is required on the inhibition of cAMP for compound **2.45** as well as the KWFQ mimics **2.05** and **2.94**. As somatostatin is a growth hormone regulator the KWQ and KWFQ mimics could also be tested as to their ability to regulate growth hormone.

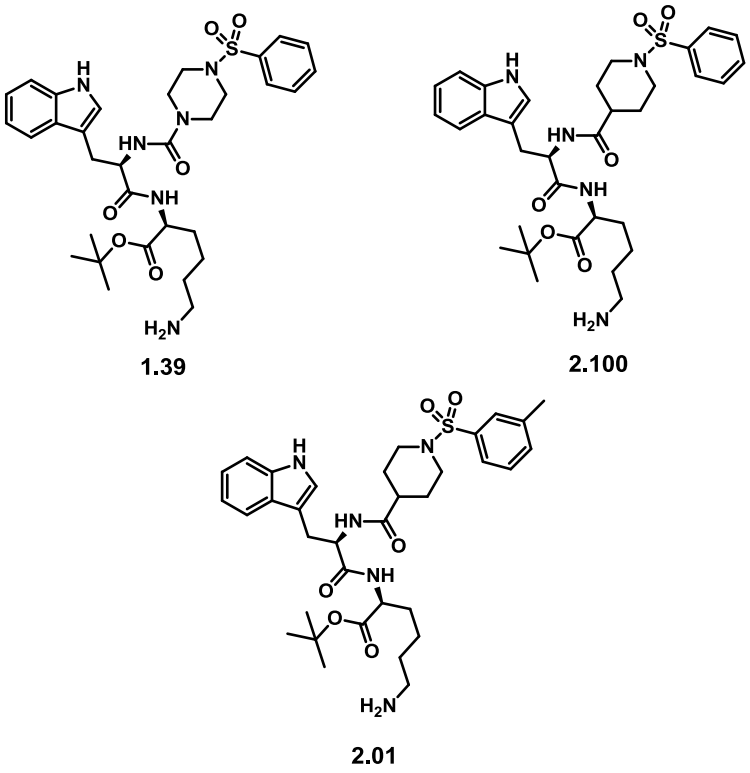


Figure 65 Compounds **1.39**, **2.01** and **2.100** synthesised by Hay and colleges[140]

2.6 References

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Chapter 3 - Lactams with Alkyl Side Chains

Within this chapter, molecules based on the acylaminolactam BSCI structure were studied to determine whether they act though functional selectivity, like the SSTR2 and hybrid ligands of Chapter 2, or whether they exert their action through an allosteric site.

3.1 Analysis of SSTR2 Binding Data of Existing BSCIs

Despite the synthesis of a range of highly potent BSCIs such as lactam **1.13** (40 pM *in vitro*) and the clinical candidate FX125L, information regarding the binding and mechanism of action of BSCIs is vital for future development and understanding of this type of drug. During the early investigations into BSCIs a large number of potential candidates were synthesised by Fox *et al*. These synthesised compounds, along with a number of natural products and natural product-derived compounds, were tested for their SSTR2 binding ability by Dr Jill Reckless of The Department of Medicine at The University of Cambridge. As part of this project, the author analysed this data so that additional conclusions could be drawn about the ligand structural requirements for SSTR2 binding and to highlight areas to be investigated further. The binding data is given as percentage inhibition of fluorescently labelled somatostatin (SS-14 FITC) from SSTR2. The data was also compared to previously reported leukocyte migration inhibition data to determine whether there was any correlation. The leukocyte migration data is reported as half maximal effective dose (ED_{50}) concentrations values of the compounds inhibition of THP-1 leukocytes migration stimulated by chemoattractant MCP-1.

Nine of the tested compounds were natural products, derivatives or existing drugs with little relation to the traditional acylaminolactam BSCI structure. Six had a binding of less than 6 %; of the remaining three, the highest binding was obtained from phenytoin sodium (Figure 66, **3.01**) with a binding of 29 %. Compound **3.01** has a five-membered succinimide ring, which in its protonated form has structural similarities to the glutarimide-containing BSCIs. Yohimbine (**3.02**) had an activity of 14 % (Figure 66), while yohimbine derivatives with an amide (**3.03**), methyl amide (**3.04**), dimethyl amide (**3.05**), carboxylic acid (**3.06**) and the stereoisomer raulwolscine (**3.07**) all had activities under 6 %. Oxymetazoline (**3.08**) (Figure 66) had an activity of 15 % and the glutarimide-containing thalidomide (**3.09**) had an activity of 4 %. In conclusion none of the natural products or known drug molecules showed high levels of activity, however they do provide a useful standard for the comparison of other molecules.

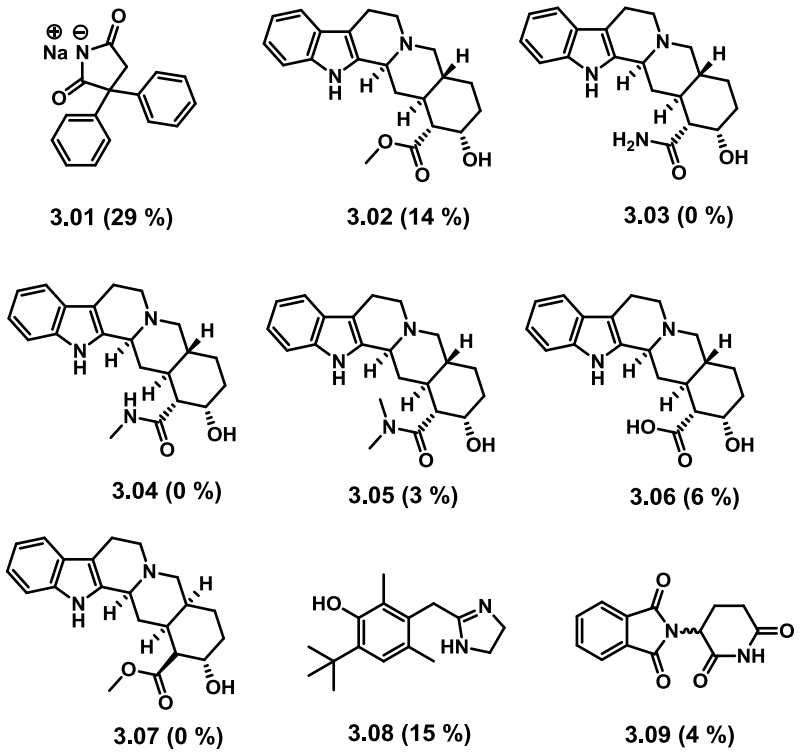


Figure 66 Compounds **3.01**-**3.09** (somatostatin binding inhibition shown in brackets, data from Dr Jill Reckless, University of Cambridge)

As previously mentioned, BSCIs are based on the tripeptide WxQ (x being any hydrophobic amino acid) and contain a hydrophobic tail group attached to a glutamine mimic. Modern BSCIs have a lactam as a glutamine mimic. A group of compounds were tested with open chain glutamine mimics. The most active of these compounds still have an activity under 17 %. As previously discussed, the errors for this assay are approximately 20 %, therefore these values may effectively be zero. The common structural similarity between these compounds is a linear eleven-carbon chain with a terminal olefin. Compound **3.10** has a dimethylated glutamic acid as the head group and shows an activity of 17 %; compound **3.11** has a glutamide head group and shows an activity of 14 % and compound **3.12** just has a methylated acid as the head group (lacking an amino acid side chain) and has an activity of 10 % (Figure 67). These compounds have

low BSCI activity, with compound **3.11** having a leukocyte migration inhibition value of 15000 nM.[45]

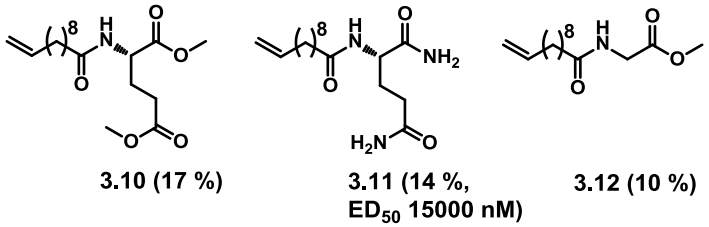


Figure 67 Compounds **3.10**-**3.12** (somatostatin binding inhibition and leukocyte migration inhibition shown in brackets, data from Dr Jill Reckless, University of Cambridge)

Of the glutarimide compounds, the most active was compound **3.13** (Figure 68) with an activity of 79 % and a leukocyte migration inhibition ED_{50} value of 66 nM.[45] Compound **1.08** (previously mentioned in Chapter 1), contains the linear eleven-carbon chain with a terminal olefin and displays similar activity to the compounds with open chain head groups with an activity of 12 %. Despite its low displacement of somatostatin from SSTR2, compound **1.08** is a more potent BSCI than glutarimide **3.13**, with an ED_{50} of 5 nM.[45] The more important compounds however were the lactams as previously stated. These retain the BSCI activity of glutarimides but with the benefit of improved metabolic stability.[46]

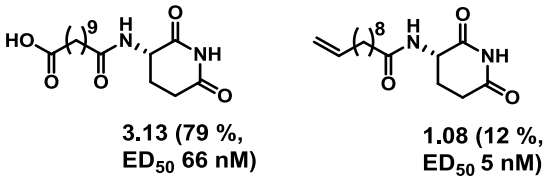
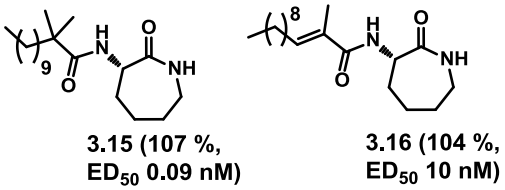


Figure 68 Compounds **3.13** and **1.08** (somatostatin binding inhibition and leukocyte migration inhibition shown in brackets, data from Dr Jill Reckless, University of Cambridge)

All of the compounds with the highest activity, regarding displacing somatostatin from SSTR2, have long carbon chain tail groups apart from one - a 6-membered

lactam with an unsubstituted phenyl ring (compound **3.14**). The general conclusion drawn from this is that a lactam, substituted with a long carbon chain or an aromatic group of some fashion, is necessary to displace somatostatin from its SSTR2 binding site. The nature of the long chain remains unclear, however some form of substitution at the 2-position appears to be necessary. For example, compound **3.15**, which contains a 2-*gem*-dimethyl group, and the mono-substituted unsaturated compound **3.16** (Figure 69) have high somatostatin displacement values of 107 and 104 % respectively, but compound **3.15** is a significantly better BSCI with an ED_{50} of 0.09 nM compared to 10 nM for compound **3.16**.[47]



3.15 (107 %, ED_{50} 0.09 nM) **3.16 (104 %, ED_{50} 10 nM)**

Figure 69 Compounds **3.15** and **3.16** (somatostatin binding inhibition and leukocyte migration inhibition shown in brackets, data from Dr Jill Reckless, University of Cambridge)

Unfunctionalised or unbranched long chain substituted lactams, such as the sulfonamide compounds **3.17-3.21** and the carboxamide, compounds **3.22-3.26**, all show much lower levels of somatostatin displacement. The 7-membered lactam sulfonamide with an 18-carbon chain, compound **3.17**, shows the highest activity at 58 % and is the only example of these classes that shows any significant activity at all. The remaining compounds, **3.18-3.21**, all had activities of less than 15 % (Figure 70).

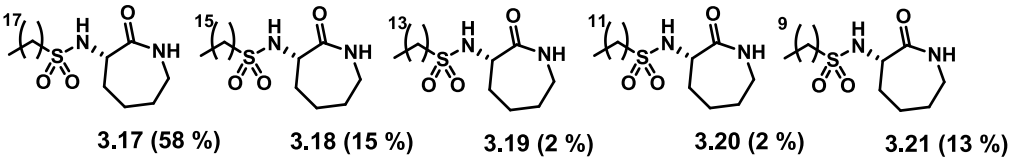


Figure 70 Compounds **3.17-3.21** (somatostatin binding inhibition shown in brackets, data from Dr Jill Reckless, University of Cambridge)

The 7-membered carboxamides all had activities of less than 18 % (Figure 71). These compounds do however show good BSCI activity. Compound **3.23** was the most potent inhibitor of leukocyte migration with an ED_{50} of 3 nM, with compounds **3.24**, **3.25** and **3.26** having ED_{50}s of 5, 15 and 50 nM respectively.[46] This indicates the unsubstituted long chain in a conformation that does not displace somatostatin, yet still manages to bind and exert BSCI activity.

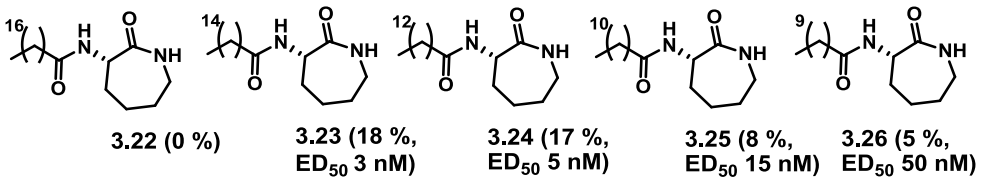


Figure 71 Compounds **3.22-3.26** (somatostatin binding inhibition and leukocyte migration inhibition shown in brackets, data from Dr Jill Reckless, University of Cambridge)

Of the compounds bearing aromatic systems the most active was compound **3.14** with 56 % displacement of somatostatin from SSTR2; this compound is also a potent BSCI with an ED_{50} of 0.09 nM (Figure 72).[202] However the errors are high at this level of inhibition. Replacing the phenyl ring with a pyridine ring, as in compound **3.27**, removes the activity completely. The 7-membered equivalent of **3.14**, compound **3.28**, has an activity of 23 % and an ED_{50} of 0.4 nM.[202] Removing the aromaticity and replacing it with one double bond, as in compound **3.29**, decreases the activity to 2 %, whilst removing the unsaturation

altogether, as in compound **3.30**, abolishes any activity. Despite their low displacement of somatostatin from SSTR2 compounds **3.29** and **3.30** have high leukocyte migration inhibition ED_{50} of 0.8 and 0.5 nM respectively.[47] This indicates that, due to the small nature of the compounds, they can bind to SSTR2 and exert their BSCI function whilst not displacing somatostatin. Other examples include the very potent compounds **3.31**, **1.13** (previously mentioned in Chapter 1) and **3.32** with ED_{50} values of 90, 40 and 70 pM respectively (Figure 72).[47] None of these inhibit somatostatin binding to SSTR2, with values of 21, 6 and 14 % respectively.

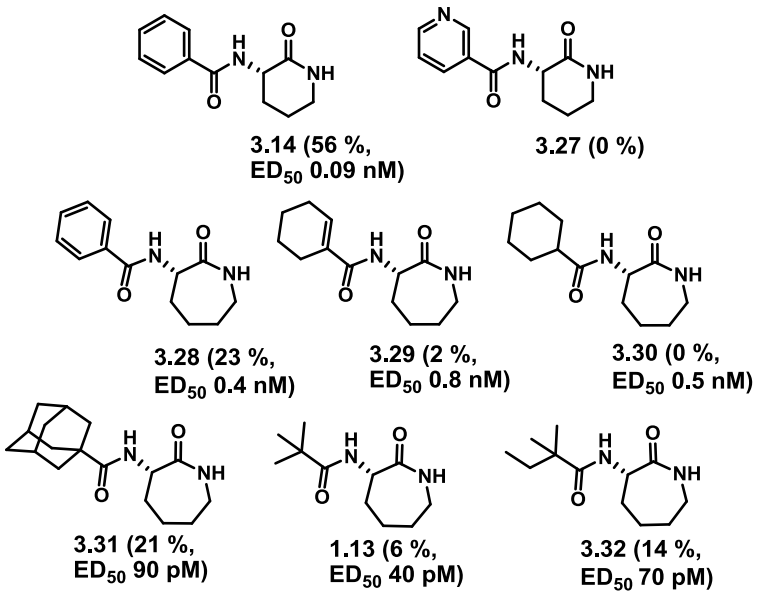


Figure 72 Compounds **3.14** and **3.27-3.30** (somatostatin binding inhibition and leukocyte migration inhibition shown in brackets, data from Dr Jill Reckless, University of Cambridge)

Comparing the importance of the lactam ring size gives mixed findings. Compound **1.09** and the analogous 7-membered (**1.11)** and 5-membered (**1.10**) compounds (previously mentioned in Chapter 1) all have similar somatostatin displacement values of 12, 2 and 0 % respectively. However their BSCI potency decreases as ring size decreases, the compounds have ED_{50}s of 40, 100 and 245

nM for compounds **1.11**, **1.09** and **1.10** respectively (Figure 73). The most active aromatic compound has a 6-membered ring, **3.14**, which displaces somatostatin from SSTR2 at 56 % compared to 23 % for the corresponding 7-membered compound **3.28**; this shows a difference in the differing ring sizes, however again errors in this region are relatively high. Their ED_{50}s are 0.4 and 0.09 nM respectively, again showing higher potency with the larger ring size of lactam.

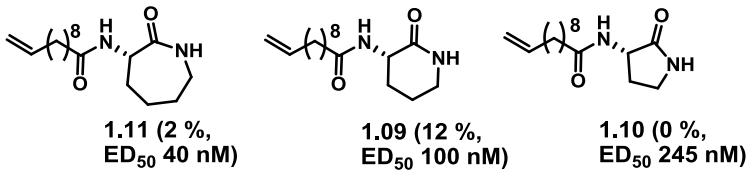
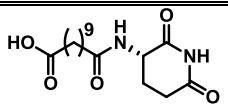
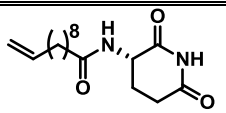
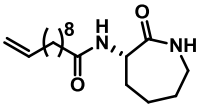
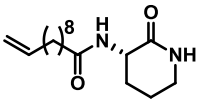
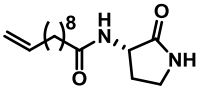
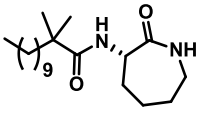


Figure 73 Compounds **1.09-1.11** (somatostatin binding inhibition and leukocyte migration inhibition shown in brackets, data from Dr Jill Reckless, University of Cambridge)

Regarding the compounds ability to displace somatostatin from SSTR2 it can be concluded from this data that lactams with a long carbon side chain are the most potent. The nature of the chain is relevant however and substitution of the 2-position as seen in compounds **3.15** and **3.16** appears to be necessary for the displacement of somatostatin from SSTR2. Those without this substitution such as the sulfonamides (**3.17-3.21**), carboxamides (**3.22-3.26**) and those with the terminal olefin (**1.09-1.11**) do not displace somatostatin from binding. A lactam with an aromatic substituent (**3.14**) has also shown reasonable high displacement of somatostatin.

From comparing the ability of the compounds to displace somatostatin from SSTR2 with their leukocyte migration inhibition it can be seen the two do not correlate (Table 5). It is not necessary for a compound to displace somatostatin from binding in order to be a good BSCI; likewise those that displace

somatostatin are not necessarily good BSCIs. The most potent BSCIs are compounds **3.31**, **3.32** and **1.13** with ED_{50} values of 90, 70 and 40 pM respectively however their displacement of somatostatin are all very low (21, 14 and 6 % respectively). Seemingly these compounds lacking the long alkyl chain substituents are able to bind to SSTR2 and exert their BSCI function while not displacing somatostatin. Compound **3.15** with substitution at the 2-position displaces somatostatin at 107 % while having a leukocyte migration inhibition value of 90 pM. However the longer chain alkyl compounds lacking substitution at the 2-position may still possess good BSCI activity as seen in the 3 nM compound **3.23**. This may be due to the nature of their side chain which allows them to fold away from the somatostatin binding domain of SSTR2.

Compound	**Structure**	**% somatostatin inhibition**	**Leukocyte migration inhibition ED_{50} (nM)**
3.13		79	66
1.08		12	5
1.11		2	40
1.09		12	100
1.10		0	245
3.15		107	0.09

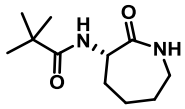
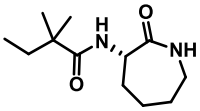
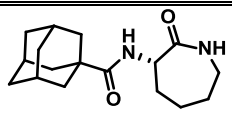
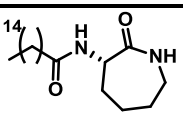
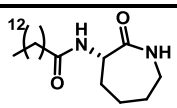
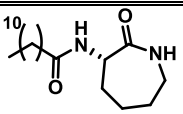
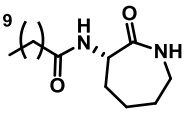
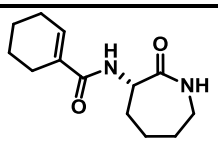
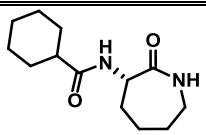
1.13		6	0.04
3.32		14	0.07
3.31		21	0.09
3.23		18	3
3.24		17	5
3.25		8	3
3.26		5	50
3.29		2	0.8
3.30		0	0.5

Table 5 % somatostatin inhibition and leukocyte migration inhibition ED_{50} (data from Dr Jill Reckless, University of Cambridge)

As a result of these findings the initial set of compounds to be investigated further will simply be acylaminolactams with an alkyl chain (ranging from a methyl group to a nonyl group), the aim of gaining more systematic information on the affect of chain length on activity, and the theory those with unsubstituted chains may be poorer at displacing somatostatin. Alkyl substituted benzoyl head groups will also be synthesised to give a hybrid of the most active compounds in

regards to displacing somatostatin from SSTR2, the long chains and the aromatic component (Chapter 4).

3.2 Lactams with Alkyl Side Chains

A series of acylamino tetrahydropyridin-2-one BSCIs compounds **3.33**-**3.40** (Figure 74) were synthesised. The 6-membered lactam head group was synthesised as previously described in Chapter 2, and was coupled to a series of commercially available acid chlorides under Schotten-Baumann conditions[185](Scheme 26). The yields are very low for the smaller compounds and gradually increase as the alkyl chain increases (Table 6). This is due to the compounds high solubility in water owing to their lack of hydrophobic groups. They are consequently washed into the aqueous layer in the work-up, reducing the isolated yield. Compounds synthesised ranged from decanoylamino- to butylanoylamino-tetrahydropyridin-2-one, all containing (*S*)-3-aminotetrahydropyridin-2-one apart from compound **3.38** which contained (*R*)-3-aminotetrahydropyridin-2-one.

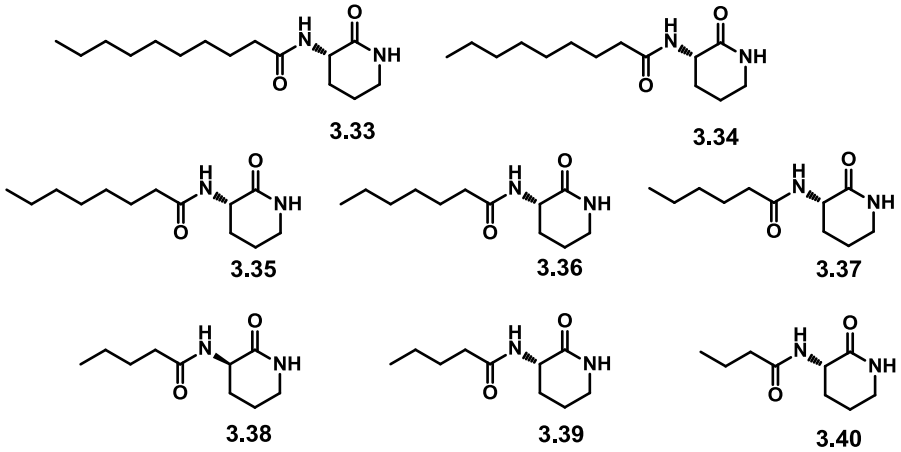
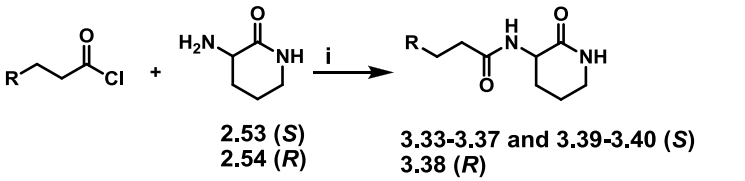


Figure 74 Compounds **3.33**-**3.40**

Compound	Yield (%)	Compound	Yield (%)
3.33	30	**3.37**	13
3.34	27	**3.38**	10
3.35	26	**3.39**	7
3.36	21	**3.40**	3

Table 6 Compounds **3.33**-**3.40** and their respective yields

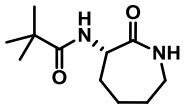


(3.33): (2.53) R= ^{n}Hept (30 % yield), **(3.34): (2.53)** R= ^{n}Hex (27 % yield),
(3.35): (2.53) R= ^{n}Pent (26 % yield), **(3.36): (2.53)** R= ^{n}Bu (21 % yield),
(3.37): (2.53) R= ^{n}Pr (13 % yield), **(3.38): (2.54)** R=Et (10 % yield),
(3.39): (2.53) R=Et (7 % yield), **(3.40): (2.53)** R=Me (3 % yield),
Reagents; (i) CH_2Cl_2/H_2O, 3 eq. Et_3N

Scheme 26 Synthesis of compounds **3.33**-**3.40**

3.3 6-Membered Exupéry Compounds

Following on from the synthesis of the longer chain acylamino tetrahydropyridin-2-ones, a series of small molecules were synthesised as potential BSCIs. At present the smallest BSCIs comprise a 6-membered or 7-membered lactam with a tertiary butyl side chain - for example the previously mentioned compound **1.13** (Figure 75). The idea was to make the smallest BSCIs possible to see how this affected their activity; an idea that was inspired by the inventor Antoine de Saint-Exupéry who said "a designer knows he has achieved perfection not when there is nothing left to add, but when there is nothing left to take away".[203] Accordingly, 5-membered, 6-membered and 7-membered lactams were synthesised with tertiary butyl, isopropyl, ethyl and methyl groups. Larger adamantane compounds were also synthesised for comparison.



1.13

Figure 75 A current small BSCI[47]

3.3.1 6-Membered Lactam via a Hydrophobic Precursor

Due to difficulties associated with recovering the small alkanoylamino tetrahydropyridin-2-one compounds after an aqueous work-up the smallest compounds were synthesised *via* a different method. As an aqueous work-up is generally necessary the attachment of the small side chain to a more hydrophobic "masked lactam" was used as a lactam precursor. The selection of the Cbz group increases hydrophobicity and allows for a neutral residue-free deprotection that does not need an aqueous work-up. Formation of the lactam was achieved by a cyclisation reaction resulting from Cbz removal *via* hydrogenolysis, which was the last step in the synthesis (Figure 76).

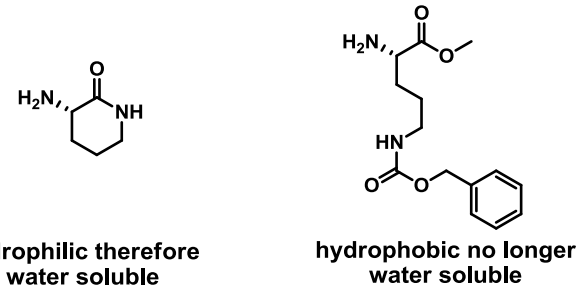
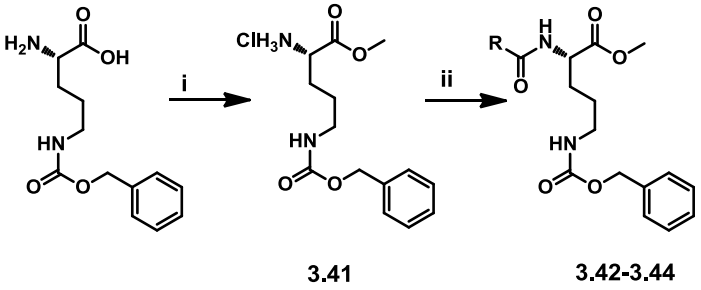


Figure 76 Lactam and "masked lactam" hydrophobic precursor

N-(Z)-Orthinine methyl ester (**3.41**) was synthesised by acid catalysed ester formation using methanolic HCl (Scheme 27). The amide bond was then formed under Schotten-Bauman conditions.[185] Isobutyryl chloride, propionyl chloride, acetyl chloride were the three acid chlorides used to give the products

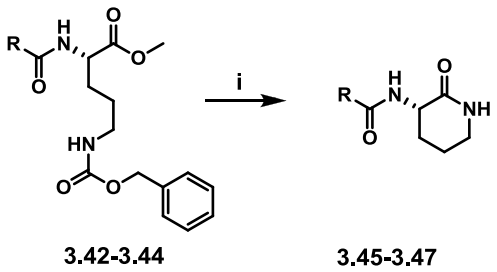
compounds **3.42-3.44**. The products were extracted with ethyl acetate and washed with *p*H 2 buffer to give higher yields then when coupled to the lactam. The yields were 83, 64 and 92 % respectively, considerably higher than the best yield of 30 % using the previous method in the synthesis of compound **3.33**.



(3.41): (99 % yield), **(3.42):** R=iPr (83 % yield),
(3.43): R=Et (64 % yield), **(3.44):** R=Me (92 % yield)
Reagents; (i) CH_3OH, AcCl, (ii) CH_2Cl_2/H_2O, 3 eq. Et_3N, RCO-Cl

Scheme 27 Synthesis of compound **3.41**-**3.44**

The target lactams was formed by a hydrogenolysis reaction which removed the Cbz group (Scheme 28). Once the Cbz group was removed, the free amine of the ornithine side chain is then able to react with the methyl ester as in the previous lactam synthesis.



(3.45): R=^{i}Pr (88 % yield), **(3.46):** R=Et (78 % yield), **(3.47):** R=Me (58 % yield)
Reagents; (i) CH_3OH, Pd/C, H_2,

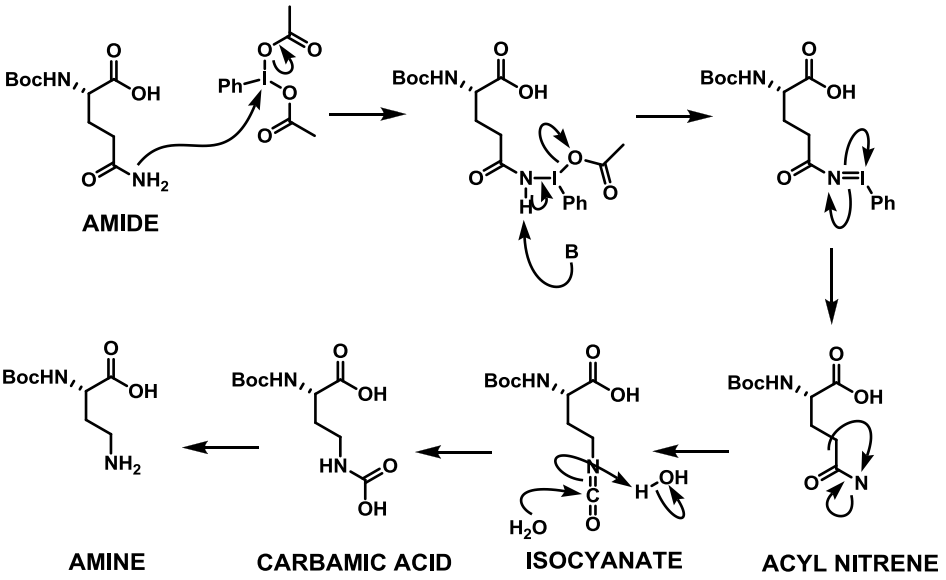
Scheme 28 Synthesis of compounds **3.45**-**3.47**

3.4 5-Membered Exupéry Compounds

The 6-membered and 7-membered ring lactams were synthesised using ornithine and lysine respectively. Diaminobutyric acid is the 5-membered lactam starting material, therefore the 5-membered lactam could be synthesised by making a methyl ester and base catalysed cyclisation.[204] The Hofmann reaction was used to synthesis diaminobutyric acid from Boc protected glutamine.

3.4.1 Hofmann Reaction

The Hofmann reaction is a method of converting an amide to an amine.[205] This method used iodobenzene diacetate to effect this transformation (Scheme 29).

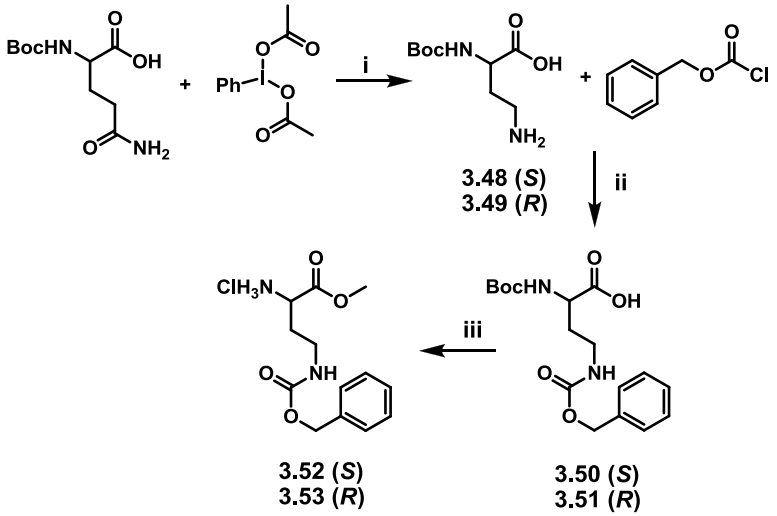


Scheme 29 Mechanism of Hofmann Reaction

The presence of the acetic acid by-product ensures the amine, once formed, is protonated, preventing it from reacting with the isocyanate to form a urea molecule.[206]

3.4.2 5-Membered Lactam via a Hydrophobic Precursor

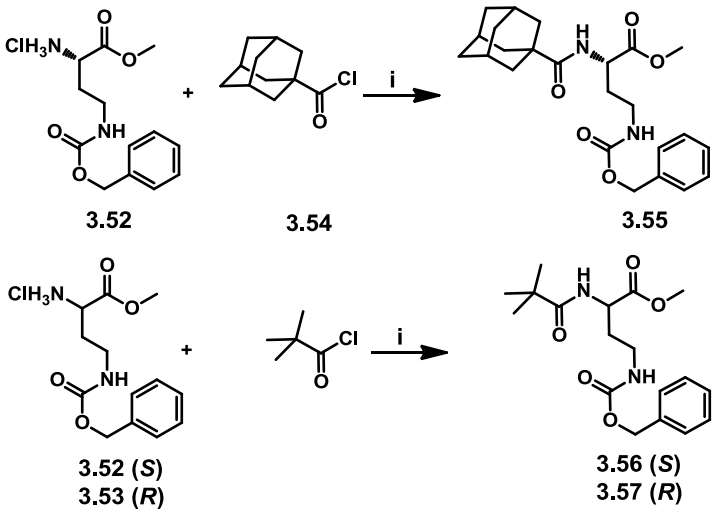
The resulting amines **3.48** and **3.49** were Cbz protected using benzyl chloroformate giving bis protected amino acids **3.50** and **3.51** (Scheme 30).[206] Methanolic acid then removed the Boc group and forms the methyl ester in one step to give compounds **3.52-3.53**.



(3.48): (*S*) (64 % yield), **(3.49):** (*R*) (98 % yield)
(3.50): (*S*) (80 % yield), **(3.51):** (*R*) (61 % yield)
(3.52) and **(3.53):** Assume quantitative yields
Reagents; (i) THF/H_2O, (ii) THF/H_2O, 1 eq. KOH, 2 eq. K_2CO_3, (iii) CH_3OH, AcCl

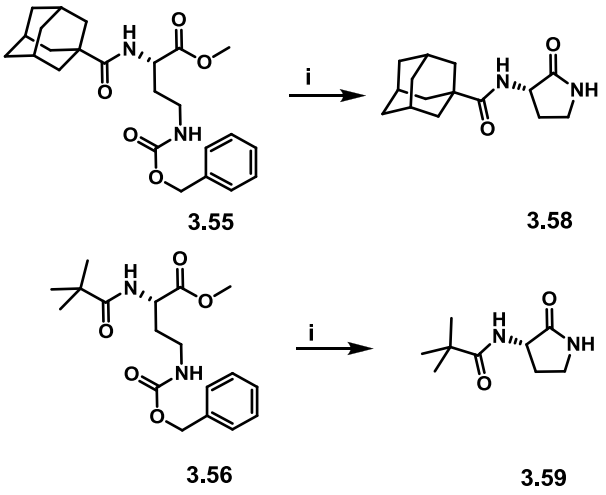
Scheme 30 Synthesis of compounds **3.48-3.53**

Amines **3.52** and **3.53** were coupled to pivaloyl chloride and adamantane carbonyl chloride to give **3.55-3.57** (Scheme 31). Adamantane carbonyl chloride (**3.54**) was synthesised from adamantane carboxylic acid with oxalyl chloride and catalytic DMF.



Scheme 31 Synthesis of compounds **3.55-3.57**

Hydrogenolysis removed the Cbz group allowing cyclisation to occur (Scheme 32). The rate of cyclisation was noticeably slower in one case, the open chain amine was recovered after hydrogenolysis. Cyclisation was induced by further stirring in methanol with gentle heating. Compounds **3.58-3.59** were successfully synthesised.

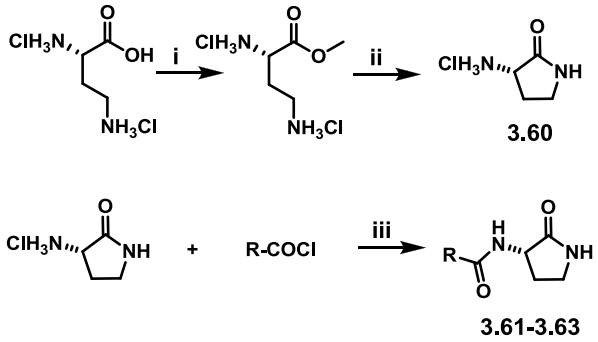


(3.58): (*S*) (61 % yield), **(3.59):** (*S*) (33 % yield)
Reagents; (i) CH_3OH, Pd/C, H_2

Scheme 32 Synthesis of compounds **3.58**-**3.59**

3.4.3 5-Membered Lactam via an Alternative Method

The very small 5-membered ring BSCIs were synthesised using a different method. The 5-membered aminolactam was synthesised in the same fashion as the 6-membered aminolactams (from the methyl ester under basic conditions) (Scheme 33). The reaction did not go to completion however the mixture of cyclised, compound **3.60** and the open chain products was carried on to the next step. Isobutyryl chloride, propionyl chloride and acetyl chloride were coupled to the mixture of products under Schotten-Baumann conditions.[185] On completion, products **3.61**-**3.63** were purified by flash column chromatography without exposure to an aqueous work-up (Scheme 33).



(3.60): Not isolated, **(3.61):** R=Me (14 % yield),
(3.62): R=Et (6 % yield), **(3.63):** R= ^{i}Pr (6 % yield)
Reagents; (i) CH_3OH, AcCl, (ii) NaOMe, (iii) CH_2Cl_2, 3 Eq. Et_3N

Scheme 33 Synthesis of compounds **3.60**-**3.63**

3.5 'Reverse' 5-Membered Lactams

Further 5-membered lactams with tertiary butyl and adamantane groups were synthesised, with the two amide bonds reversed (Figure 77). This gave compounds with the same chemical formula and molecular weights to see the affect of the amide bond orientation.

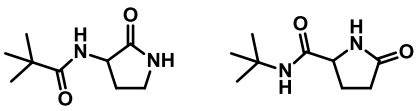
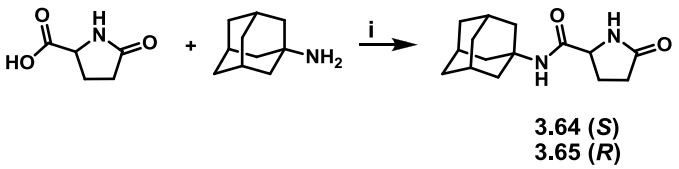


Figure 77 5-Membered lactams and 'reverse' compounds

3.5.1 Reverse Molecules from Pyroglutamic Acid

The 'reverse' lactams were synthesised by coupling pyroglutamic acid to adamantamine and tertiary butylamine using HATU as a coupling reagent. The compounds from adamantamine (**3.64**-**3.65**) were synthesised successfully, however the yields were quite low. This is most likely again due to loss of a certain amount of the product during the aqueous work-up.



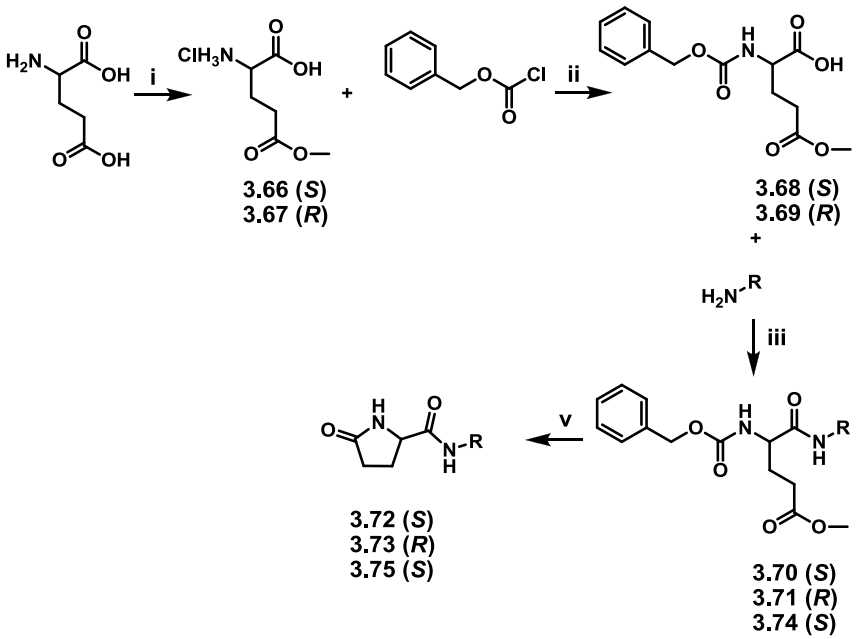
3.64 (*S*)
3.65 (*R*)

(3.64): (*S*) (48 % yield), **(3.65):** (*R*) (24 % yield)
Reagents; (i) CH_2Cl_2, HATU, 3 eq. Et_3N

Scheme 34 Synthesis of compounds **3.64** and **3.65**

3.5.2 'Reverse' Molecules via a Hydrophobic Precursor

This method was not successful for the 'reverse' lactams with a tertiary butyl group due to their increased solubility in water. A hydrophobic lactam precursor was therefore synthesised for the 'reverse' lactams. Glutamic acid methyl ester was synthesised from glutamic acid using methanolic HCl (Scheme 35, **3.66**, **3.67**).[206] The amine was Cbz protected with benzyl chloroformate to give acids **3.68** and **3.69**. The acid was then coupled to tertiary butyl amine with HATU to give compounds **3.70** and **3.71**. The lactam was formed by removing the Cbz in another hydrogenolysis reaction to give lactams **3.72** and **3.73**. Compound **3.74**, the isopropyl lactam precursor equivalent, was synthesised in the same fashion. Isopropylamine was coupled to acid **3.68**, deprotection *via* hydrogenolysis and subsequent cyclisation gave the 'reverse' lactam **3.75**.



(3.66): (*S*) (96 % yield), **(3.67):** (*R*) (96 % yield), **(3.68):** (*S*) (27 % yield), **(3.69):** (*R*) (73 % yield), **(3.70):** (*S*) R=^{t}Bu, (33 % yield), **(3.71):** (*R*), R=^{t}Bu, (32 % yield), **(3.72):** (*S*), R=^{t}Bu, (45 % yield), **(3.73):** (*R*), R=^{t}Bu, (24 % yield), **(3.74):** (*S*), R=^{i}Pr, (46 % yield), **(3.75):** (*S*), R=^{i}Pr, (72 % yield)
Reagents; (i) CH_3OH, Me_3SiCl, (ii) dioxane/H_2O, 3 eq. $NaHCO_3$, (iii) CH_2Cl_2, 3 eq. Et_3N, (v) CH_3OH, Pd/C, H_2

Scheme 35 Synthesis of compounds **3.66**-**3.73**

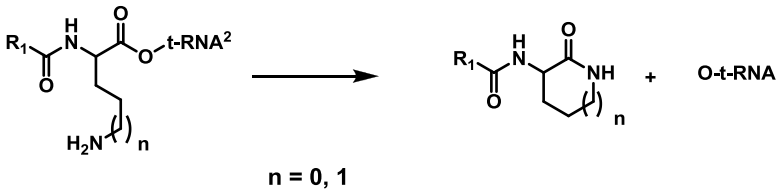
3.6 7-Membered Exupéry Compounds

The tertiary butyl and adamantane 7-membered lactams (compounds **1.13** and **3.31**) had been previously synthesised by the Fox group.[47] To complete the series, the isopropyl, ethyl and methyl lactams were synthesised. These could not be synthesised *via* a hydrophobic precursor due to the difficulty in cyclisation of 7-membered lactams. The necessary heating under reflux conditions would cause epimerisation of the sterocentre as shown in Chapter 2.

3.6.1 Different Rates of Lactam Cyclisation

6-Membered lactams cyclise at the fastest rate, followed by 5-membered and finally 7-membered. This may explain the absence of diaminobutryic acid and ornithine as amino acids coded for by t-RNA. Both could undergo spontaneous

lactamisation (Scheme 36). In addition to this, diaminobutyric acid can undergo reversible acyl transfer reactions.[207, 208]



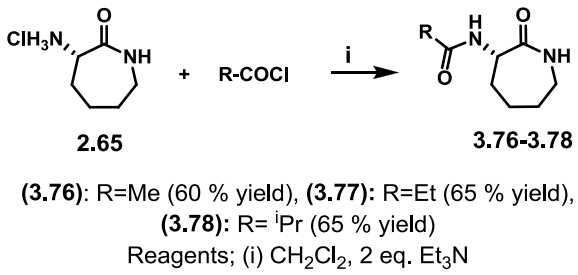
Scheme 36 Ornithine may undergo spontaneous lactamisation in a peptide chain

The rate of lactam cyclisation differs to that of saturated heterocycles, where 5-membered rings form faster than 6-membered rings. The smaller rings form faster on entropic grounds, as there are fewer bonds to align. The transition state for the tetrahedral intermediates of the 6-membered lactam is close in structure to a stable chair conformations. They result in a lactam in a half chair conformation with the amide bond lying flat. The transition state for the tetrahedral intermediate of the 5-membered lactam is less stable not being able to resemble a chair conformation. The 5-membered ring forms slower in this case due to enthalpic reasons explaining the slower rate of cyclisation seen. The 5-membered lactam ring is more strained, but by allowing the amide to lie flat strain is partially relieved by slight puckering of the ring. The 7-membered lactams close *via* a pseudo-chair conformation transition state. On deprotection of the Cbz protected hydrophobic lactam precursors the 6-membered lactams form immediately. The 5-membered lactams do not always form without further heating. On synthesis of the free lactams the 6-membered cyclises in 2 hours at 0 °C. The 5-membered lactam was kept at 0 °C to prevent epimerisation under the basic conditions. The reaction however only proceeded to 50 % completion even

after 48 hours. The 7-membered lactam required heating under reflux conditions for at least 48 hours for complete ring closure to happen.

3.6.2 7-Membered Lactam Synthesis

The synthesis of the 7-membered Exupéry compounds therefore started with (*S*)-3-amino-azepan-2-one.HCl **2.65**. Isobutyryl chloride, propionyl chloride and acetyl chloride were all coupled to lactam **2.65**. Two equivalents of triethylamine were used to neutralise the salt and neutralise the HCl produced (Scheme 37). The addition of diethyl ether to the reaction caused this triethylamine hydrogen chloride to precipitate. The filtrate was reduced *in vacuo* and purified by flash column chromatography to give lactams **3.76-3.78**.[209]



Scheme 37 Synthesis of compounds **3.76-3.78**

3.7 Biological Results

3.7.1 SSTR2 Binding Data

BSCIs bind to SSTR2, as shown by their displacement of somatostatin from the receptor. The exact location of the binding site is however unknown. It could be the same binding site as somatostatin or an allosteric binding site. The SSTR2 binding data was again determined by Tilly Sharp at Total Scientific (Babraham Research Campus, Cambridge) using FP by exactly the same method as for the compounds in Chapter Two. The data is given as percentage inhibition of SS-14 FITC from SSTR2. None of the compounds tested (Figure 78) showed any

significant displacement of somatostatin from SSTR2 at a concentration of 100 μM (Table 7). These results correlate to those of the 7-membered lactams (**3.22-3.26**). The data for the Exupéry compounds correlates with the smaller compounds seen at the start of the chapter (**3.29-3.32**) (Table 8) - none of the six ring compounds with a methyl, ethyl or isopropyl group or the 'reverse' compounds show any displacement of somatostatin.

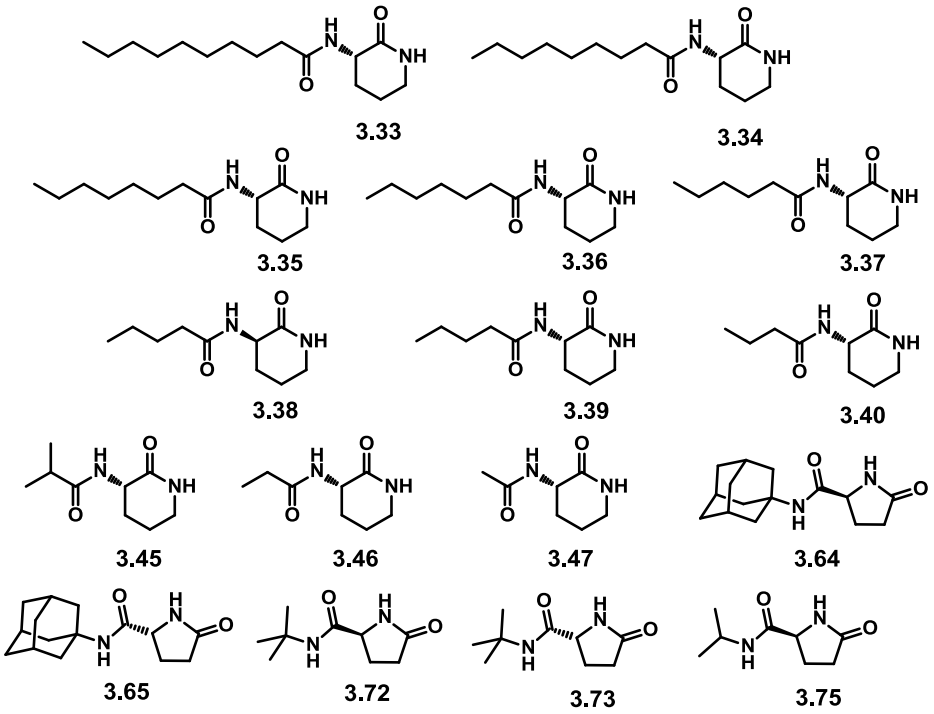


Figure 78 Compounds tested for SSTR2 binding

Compound	% Somatostatin Inhibition	Compound	% Somatostatin Inhibition
3.33	2	**3.37**	-20
3.34	5	**3.38**	-2
3.35	10	**3.39**	1
3.36	-12	**3.40**	-3

Table 7 % at 1 nM of SS-14 FITC from SSTR2 (data from Tilly Sharp at Total Scientific)

Compound	% Somatostatin Inhibition	Compound (reverse)	% Somatostatin Inhibition
3.45	0	**3.64**	-1
3.46	0	**3.65**	-1
3.47	3	**3.72**	-1
		3.73	-8
		3.75	-8

Table 8 % inhibition at 1 nM of SS-14 FITC from SSTR2 (data from Tilly Sharp at Total Scientific)

As previously discussed the errors associated with the measurement in this assay are about 20 %. The errors associated with the concentration are lowest around 0 and 100 %. All the data is around 0 % with the highest being only 10 % so therefore the errors are minimal.

3.7.2 Leukocyte Migration Assay

Leukocyte migration data was again determined by Dr Jill Reckless of The Department of Medicine, The University of Cambridge. The data is given as percentage inhibition of neutrophil migration at given concentrations. The results for the Exupéry compounds (Figure 79) are shown in Table 9 and Table 10. They include the eleven synthesised compounds and four compounds previously synthesised by the group (labelled with an asterisk, compounds **1.13**, **3.31** and **3.79**-**3.80**).[47] They were tested at concentrations of 1 µM and 1 nM. Figure 75 shows the 1 µM results in red and 1 nM results in blue. The results show that of the fifteen compounds tested (Figure 79), the majority had excellent activity (over 98 %). The least active compounds were the three with the methyl group sidechain - even at a concentration of 1 µM, the percentage inhibition is as low as 47 % for the 5-membered compound (**3.61**) and approximately 75 % for the 6-membered and 7-membered compounds (**3.47** and **3.76**). These values drop to 25

% for **3.61** at a concentration of 1 nM and approximately 50 % for the **3.47** and **3.76**. Continuing the trend, the next least active compounds were the next smallest - the three compounds with the ethyl group (**3.62**, **3.46** and **3.77**). These compounds showed high activity at a concentration of 1 μM, all over 98 %. However at a concentration of 1 nM the activity dropped significantly to 72, 86 and 92 % for the 7-memberd, 6-membered and 5-membered compounds respectively. The remaining compounds with the isopropyl, tertiarybutyl and adamantane groups all have activity over 98 % at both concentrations. These findings indicate that for highest BSCI activity the lactams require a branched side chain. The ring size seems to be optimal at either a 6-membered or 7-membered lactam. They show there are number of potential structures for successful BSCIs. Pharmacokinetic studies have shown the adamantane structure is easily metabolised.[47]

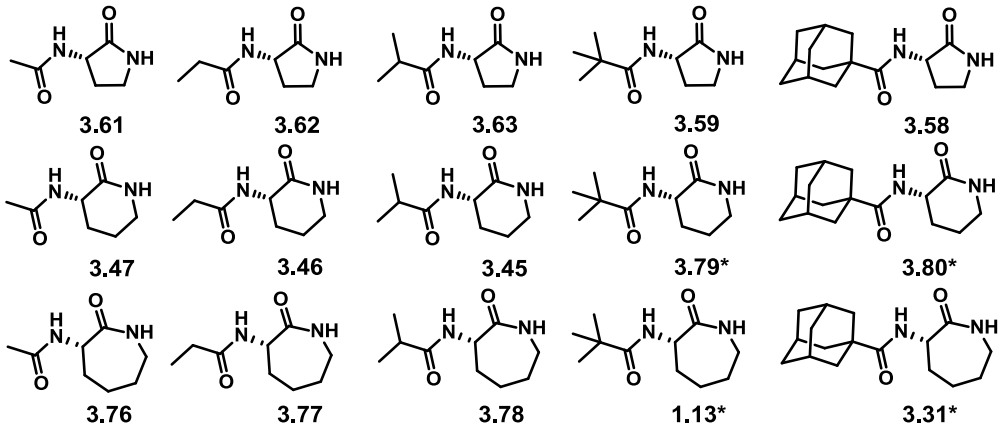


Figure 79 Exupéry compounds

5 Ring Compound	% Inhibition ± Error	6 Ring Compound	% Inhibition ± Error	7 Ring Compound	% Inhibition ± Error
3.61	47 ± 3	**3.47**	74 ± 7	**3.76**	75 ± 6
3.62	104 ± 17	**3.46**	105 ± 4	**3.77**	98 ± 37
3.63	102 ± 5	**3.45**	102 ± 6	**3.78**	104 ± 21
3.59	110 ± 11	**3.79***	101 ± 30	**1.13***	106 ± 19
3.58	103 ± 13	**3.80***	99 ± 23	**3.31***	111 ± 31

Table 9 % inhibition of neutrophil migration a 1 µM, errors represent 1 SD (data from Dr Jill Reckless, University of Cambridge)

5 Ring Compound	% Inhibition ± Error	6 Ring Compound	% Inhibition ± Error	7 Ring Compound	% Inhibition ± Error
3.61	25 ±6	**3.47**	52 ±7	**3.76**	55 ±8
3.62	92 ±23	**3.46**	86 ±13	**3.77**	72 ±10
3.63	100 ±21	**3.45**	110 ±20	**3.78**	109 ±16
3.59	104 ±9	**3.79***	104 ±30	**1.13***	107 ±12
3.58	109 ±30	**3.80***	107 ±22	**3.31***	108 ±21

Table 10 % inhibition of neutrophil migration a 1 nM, errors represent 1 SD (data from Dr Jill Reckless, University of Cambridge)

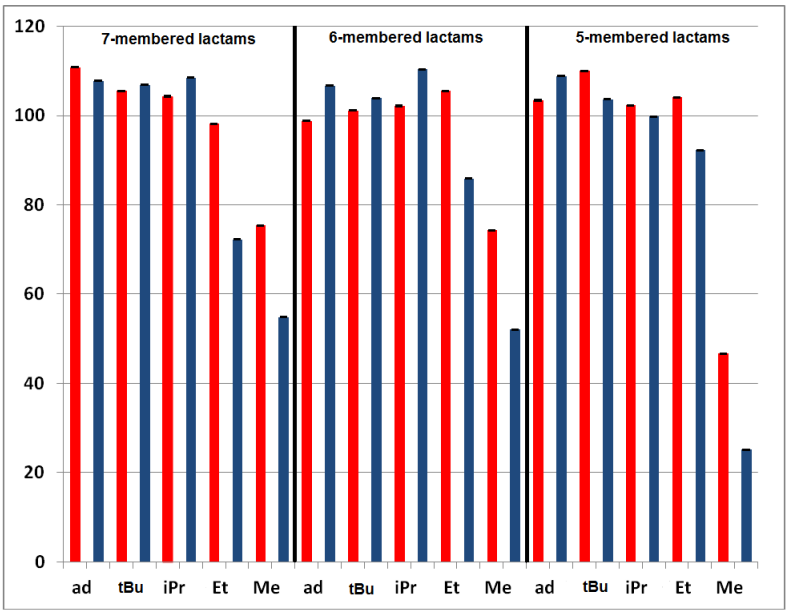


Figure 80 % inhibition of neutrophil migration, at 1 µM red and 1 nM blue

The five 'reverse' compounds (Figure 81) were all tested at a concentration of 1 µM (Table 11). All showed inhibition over 60 %, however this is relatively low compared to the non-'reverse' compounds at 1 µM. The most potent compound was the isopropyl substituted (*S*)-lactam, followed by the tertiary butyl substituted (*S*)-lactam and the adamantane substituted (*S*)-lactam. The compounds with (*R*)-stereochemistry were the least potent, again following the same pattern with the tertiary butyl substituted compound being more potent than the adamantane substituted compound. The 'reverse' isopropyl, tertiary butyl and adamantane substituted (*S*)-lactams have potencies of 90, 81 and 78 % respectively, compared to the analogous Exupéry isopropyl, tertiary butyl and adamantane substituted 5-membered (*S*)-lactams with potencies of 102, 110 and 103 %. These findings show reversing the amide bonds in the lactams does not reduce BSCI activity completely however it does significantly decrease it.

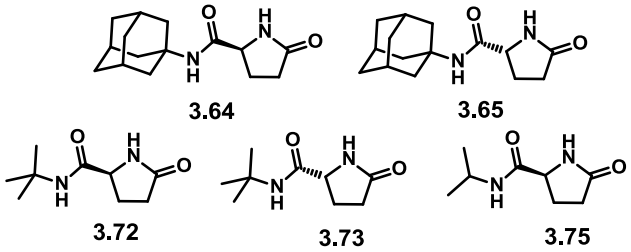


Figure 81 Reverse compounds **3.64**-**3.65**, **3.72**-**3.73** and **3.75**

Compound	**% Inhibition ± error**
3.64	78 ± 17
3.65	64 ± 9
3.72	81 ± 31
3.73	76 ± 23
3.75	90 ± 29

Table 11 % inhibition of neutrophil migration at 1 μM, errors represent 1 SD (data from Dr Jill Reckless, University of Cambridge)

3.8 Conclusions

The SSTR2 binding data of the compounds synthesised in this chapter correlates with the results seen from the data analysed at the start of the chapter. Compounds **3.33-3.40**, the 6-membered acylaminolactams with the linear alkyl substituents showed no inhibition of the binding of somatostatin at SSTR2. This was seen in the analogous 7-membered carboxamides (**3.22-3.26**), the sulfonamides (**3.17-3.21**) and those with a terminal olefin (**1.09-1.11**). The nature of the long chain means the conformation does not block the binding of somatostatin.

The Exupéry and 'reverse' compounds show no displacement of somatostatin which again correlates with the results seen from the smaller compounds at the start of this chapter (compounds **3.29**-**3.32**). Some of these compounds are potent BSCIs, showing it is not necessary for the compound to displace somatostatin to act as potent BSCIs. Preventing the binding of somatostatin could have detrimental biological effects due to the fact it has a number of functions within the body including growth hormone regulation. The Exupéry and 'reverse' BSCIs do not prevent somatostatin binding which is a desirable property as they will not act as somatostatin regulators thus yielding unwanted side effects.

The BSCI data shows that there are a number of Exupéry compounds which are potent inhibitors of leukocyte migration, presenting a number of potential clinical candidates. Modifying the compounds by reversing the amide bonds do not improve their potency therefore compounds based on the Exupéry model are the best. Other biological data being similar then factors to consider in choosing a

clinical candidate would be the ease of synthesis. The 6-membered lactams are at present the easiest to synthesise due to the fact they cyclise under mild conditions and retain their stereochemical integrity.

3.9 References

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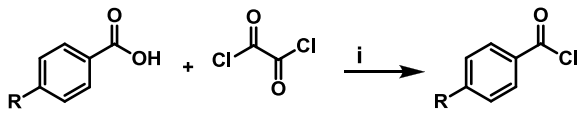
Chapter 4 - Lactams with Alkyl Substituted Aromatic Side Chains

4.1 Introduction

The initial analysis of previously synthesised compounds in Chapter 3 highlighted that long chain or benzoyl BSCIs display high binding to the receptor SSTR2. To include both of these motifs, a series of benzoyl and benzenesulfonyl lactams were synthesised with different lengths of alkyl chains in the *para*, *meta* and *ortho* position.

4.2 4-Carboxy Compounds

Twenty 4-alkylbenzoyl lactams were synthesised, comprising of 6-membered and 7-membered lactams of both (*S*)- and (*R*)-stereochemistry and one of five alkyl substituents (ethyl, butyl, *tert*-butyl, hexyl and octyl). Five commercially available acids, 4-ethylbenzoic acid, 4-^{n}butylbenzoic acid, 4-^{t}butylbenzoic acid, 4-^{n}hexylbenzoic acid and 4-^{n}octylbenzoic acid, were made into the corresponding acid chlorides **4.01**-**4.05**. They were synthesised using oxalyl chloride in dichloromethane with catalytic DMF as described in Chapter 2 (Scheme 38).

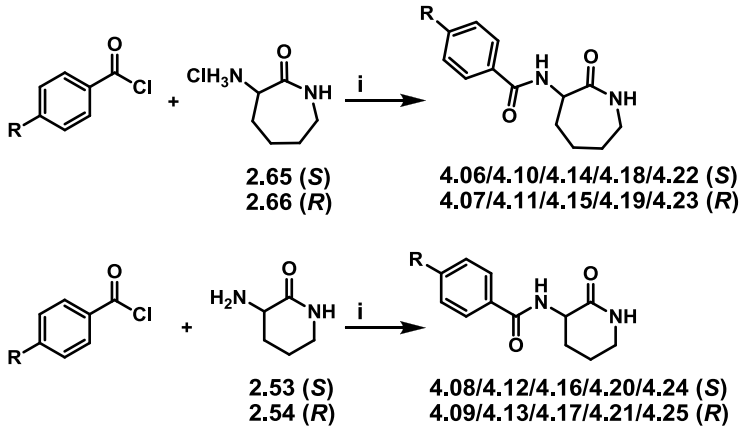


4.01-4.05

(4.01): R=Et, **(4.02):** R=nBu, **(4.03):** R=tBu, **(4.04):** R=nHex, **(4.05):** R=nOct
Reagents; (i) CH_2Cl_2, DMF

Scheme 38 Synthesis of compounds **4.01**-**4.05**

The acid chlorides were coupled to the lactams under Schotten-Baumann[185] conditions (Scheme 39, **4.06**-**4.25**).

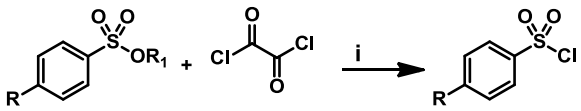


(4.06): (2.65) R=Et (36 % yield), **(4.07): (2.66)** R=Et (22 % yield)
(4.10): (2.65) R=nBu (18 %yield), **(4.11): (2.66)** R=nBu (48 % yield)
(4.14): (2.65) R=tBu (50 % yield), **(4.15): (2.66)** R=tBu (50 % yield)
(4.18): (2.65) R=nHex(48 % yield), **(4.19): (2.66)** R=nHex (69 % yield)
(4.22): (2.65) R=nOct (4 % yield), **(4.23): (2.66)** R=nOct (10 % yield)
(4.08): (2.53) R=Et (37 % yield), **(4.09): (2.66)** R=Et (45 % yield)
(4.12): (2.53) R=nBu (16 % yield), **(4.13): (2.66)** R=nBu (40 % yield)
(4.16): (2.53) R=tBu (53 % yield), **(4.17): (2.66)** R=tBu (38 % yield)
(4.20): (2.53) R=nHex (63 % yield), **(4.21): (2.66)** R=nHex (35 % yield)
(4.24): (2.53) R=nOct (83 % yield), **(4.25): (2.66)** R=nOct (25 % yield)
Reagents; (i) CH_2Cl_2/H_2O, 3 eq. Et_3N

Scheme 39 Synthesis of compounds **4.06**-**4.25**

4.3 4-Sulfonyl Compounds

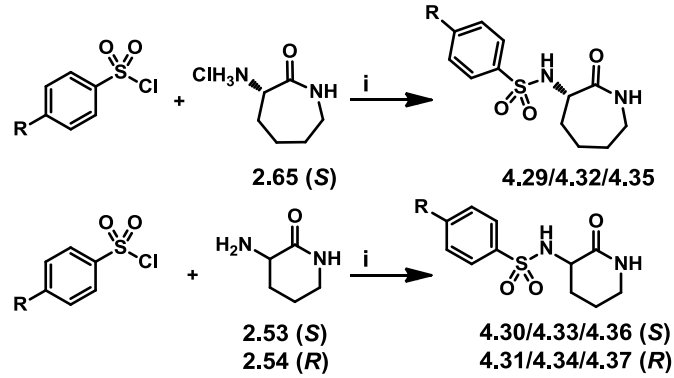
A series of 4-alkylbenzenesulfonyl lactams were synthesised, again comprising of the 6-membered and 7-membered lactams of both (*S*)- and (*R*)-stereochemistry, with the same five alkyl substituents as used for the 4-carboxy compounds and (ethyl, butyl, *tert*-butyl, hexyl and octyl). 4-*tert*-Butylbenzenesulfonylchloride (**4.27**) was commercially available, whilst 4-ethylbenzenesulfonic acid and 4-octylbenzenesodiumsulfonate were converted into the corresponding sulfonyl chlorides, compounds **4.26** and **4.28**. (Scheme 40).



(4.26): R=Et, R_1=H, **(4.28):** R=nOct, R_1=Na
Reagents; (i) CH_2Cl_2, DMF

Scheme 40 Synthesis of compounds **4.26** and **4.28**

The sulfonylchlorides **4.26**-**4.28** were coupled to the lactams in a Schotten-Baumann[185] reaction, this time the acid chloride is a sulfonyl chloride (Scheme 41).



2.65 (*S*) **4.29/4.32/4.35**

2.53 (*S*) **4.30/4.33/4.36 (*S*)**
2.54 (*R*) **4.31/4.34/4.37 (*R*)**

(4.29): (2.65) R=Et (19 % yield),**(4.30): (2.53)** R=Et (24 % yield),
(4.31): (2.54) R=Et (21 % yield), **(4.32): (2.65)** R=tBu (25 % yield),
(4.33): (2.53) R=tBu (30 % yield), **(4.34): (2.54)** R=tBu (43 % yield)
(4.35): (2.65) R=nOct (59 % yield),**(4.36): (2.53)** R=nOct (63 % yield),
(4.37): (2.54) R=nOct (45 % yield)
Reagents; (i) CH_2Cl_2/H_2O, 3 eq. Et_3N

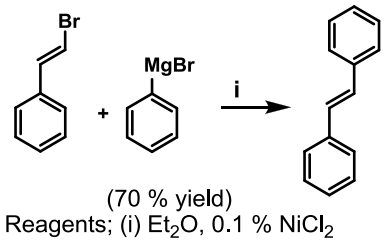
Scheme 41 4-Sulfonyl compounds **4.29**-**4.37**

For the 4-butyl and 4-hexylbenzenesulfonyl chlorides, the corresponding acids were not commercially available. These were instead synthesised from 4-chlorobenzenesulfonic acid. This required a reaction involving the formation of a carbon-carbon bond.

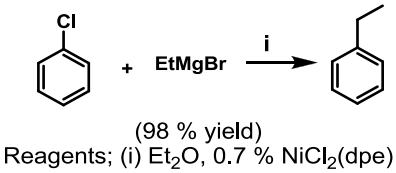
4.3.1 Methods of C-C Bond Formation

In organic synthesis carbon-carbon bond forming is one of the most important reactions. Catalytic amounts of transition metals salts were shown to induce reactions of Grignard reagents and organic halides by Kharasch as far back as the

1940s.[210] In 1972 Corriu[211] (Scheme 42) and Kumada[212] (Scheme 43) independently reported the nickel catalysed cross-coupling of Grignard reagents and aryl or alkenyl halides. This led the way into a huge amount of research into metal catalysed cross-coupling reactions, resulting in a number of commonly used reactions, valued for their mild reaction conditions and tolerance of functional groups.

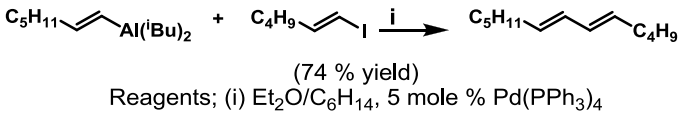


Scheme 42 Corriu's nickel catalysed coupling [211]

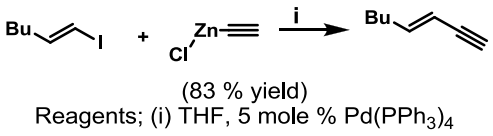


Scheme 43 A Kumada cross-coupling[212]

After his discovery in 1972 Kumada developed the Kumada cross-coupling reaction, in which palladium or nickel-phosphine complexes catalyse the reaction of Grignard or organolithium compounds with aryl chlorides, bromides or iodides. Due to the electropositive nature of magnesium or lithium, base sensitive groups are not tolerated.[213] The Negishi cross-coupling uses organozinc compounds as a less electropositive metal version of the Kumada. In 1976 Negishi reported the first nickel catalysed reaction of organoaluminium compounds (Scheme 44) and aryl or alkenylhalides.[214] The most successful however proved to be organozinc compounds with catalytic palladium (Scheme 45).[215]

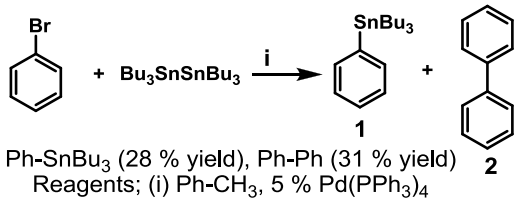


Scheme 44 A Negishi cross-coupling using an organoaluminium compound [214]

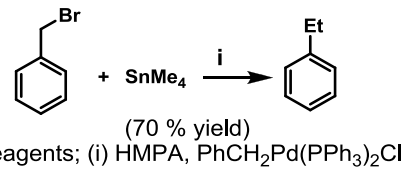


Scheme 45 A Negishi cross-coupling using an organozinc compound[215]

The Stille cross-coupling is the palladium catalysed reaction of organostannane compounds with aryl halides. The first of these was published by Eaborn in 1976[216] (Scheme 46), in which organostannanes (formed *in situ* from distanannes) coupled with aryl halides. Stille realised that organostannane compounds and aryl halides could be coupled and pioneered the use of this method (Scheme 47).[217] Advantages include their tolerance to a high number of functional groups and that they are easily prepared and stored, due to their stability to moisture and oxygen. They are, however, toxic and traces of tin are difficult to remove from the product.

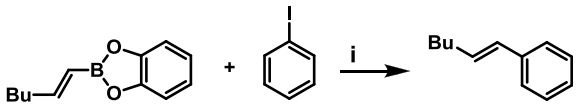


Scheme 46 Eaborn's use of organostannanes



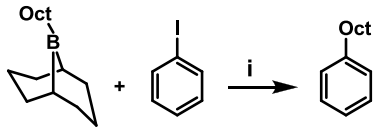
Scheme 47 A Stille cross-coupling [217]

Suzuki and Miyaura reported the first example of what came to be known as the Suzuki reaction in 1979 (Scheme 48). They showed that aryl halides would react with alkenylboranes in the presence of catalytic palladium and base to give the coupled product.[218] Advantages include the non-toxic boron by-products, which are easily separated from the product. Aryl chlorides however are much less reactive than the corresponding aryl iodides and bromides.[219] In 1986 Suzuki and Miyaura reported the successful cross-coupling of aryl or alkenyl halides with alkyl boranes - the β-alkyl Suzuki-Miyaura reaction (Scheme 49). Previously these reactions had been unsuccessful due to problems associated with β-hydride elimination. By optimising the palladium catalyst with a bis(diphenylphosphino)ferrocene (dppf) ligand, $PdCl_2$(dppf) proved to be efficient enough to react at a faster rate than unwanted side reactions could occur.[220]



(100 % yield)
Reagents; (i) C_6H_6, $Pd(PPh_3)_4$, EtONa

Scheme 48 A Suzuki cross-coupling[218]

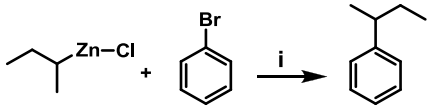


(100 % yield)
Reagents; (i) THF, 3 % $Pd(Cl_2)$dppf, NaOMe

Scheme 49 A Suzuki-Miyaura cross-coupling[220]

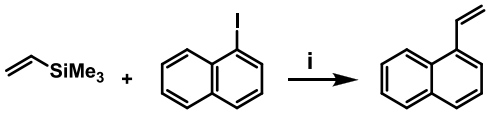
In 1984 Hayashi reported the use of the same catalyst $PdCl_2$(dppf) for the successful cross-coupling of primary or secondary alkyl Grignard and alkylzinc reagents with aryl halides (Scheme 50).[221] In 1988 Hiyama reported the use of organosilicon

compounds, which reacted with aryl, allyl or alkenyl halides with a palladium catalyst (Scheme 51). Previously it had been thought the carbon-silicon bond was too strong to be broken in a reductive elimination and these reactions had not been successful. Hiyama uses tris(diethylamino)sulfonium difluorotrimethylsilicate (TSAF) as a source of fluoride anions to cleave the carbon silicon bond and facilitate transmetallation.[222] More recently, fluoride free couplings have been achieved. Disiloxanes, which are in equilibrium with the active silanolate species, cross-couple with arylhalides catalysed by palladium in the presence of base.[223]



(98 % yield)
Reagents; (i) THF, 1 % $Pd(Cl_2)dppf$

Scheme 50 A Hayashi cross-coupling[221]

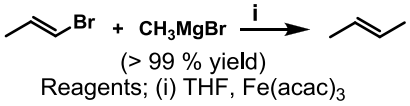


(98 % yield)
Reagents; (i) HMPA, 2.5 % C_3H_5PdCl, TSAF

Scheme 51 A Hiyama cross-coupling[222]

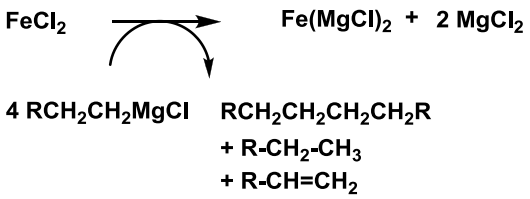
Furstner has reported the use of iron as the catalyst in the cross coupling of aryl halides and Grignard reagents. Iron boasts a number of advantages - it is both cheaper and less toxic than metals such as tin, nickel and palladium. Iron catalysts have been successful without the need for ancillary ligands to render them sufficiently active like other metals. Finally, iron catalysts have been shown to be more successful in reactions with aryl chlorides, which are cheaper alternatives to

aryl bromides or iodides in which palladium and nickel are not. Iron was first reported as a cross coupling catalyst by Kochi in 1971 (Scheme 52).[224, 225]

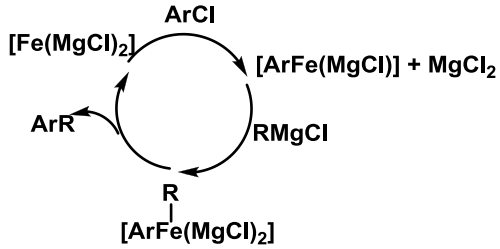


Scheme 52 Kochi's use of an iron catalyst[226]

Little follow up work has been carried out; however what was done focused on alkenyl halides.[227] The first reported heteroaryl bromide and Grignard iron catalysed coupling was reported by Figadere.[228] Furstner however reported the first aryl chloride and Grignard reagent coupling. Furstner has shown that iron catalyses the reaction of aryl halides with Grignard reagents and, in contrast to other cross-coupling reactions, aryl chlorides react most successfully - another advantage owing to the relatively low cost of aryl chlorides compared to aryl bromides and iodides. These studies showed that alkyl Grignard reagents were much more successful than aryl, allyl or alkenyl reagents. $FeCl_2$ reacts with four equivalents of Grignard reagent to give $[Fe(MgCl)_2]$ as shown in Scheme 53.[229] This gives an Fe^{-II} complex which, due to its high nucleophilic character, can oxidatively insert into an aryl halide bond to give an Fe^0 complex. Another equivalent of the Grignard reagent reacts with the iron and reductive elimination yields the product (Scheme 54).



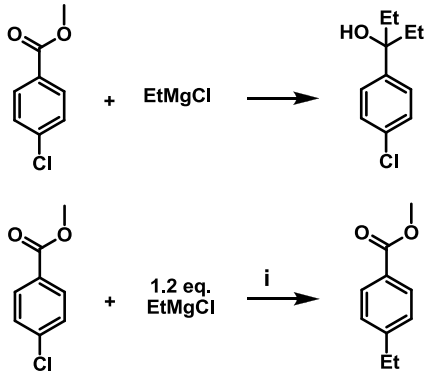
Scheme 53 Reaction of $FeCl_2$ with 4 equivalents of Grignard reagent



Scheme 54 Mechanism for Fe catalysed cross-coupling

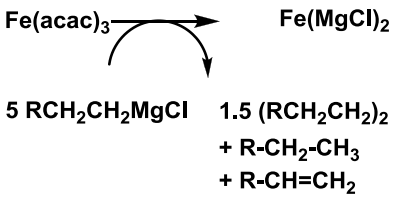
4.3.2 Use of Fe Cross-Coupling Reaction

The reaction used to synthesise the target 4-alkylbenzenesulfonic acids and 3- and 2-alkylbenzoic acids was Furstner's iron cross-coupling reaction. Conventionally reacting methyl-4-chlorobenzoate with a Grignard reagent such as EtMgCl would give a tertiary alcohol. The ethyl anion acts as a nucleophile and attacks the ester resulting in the loss of the methyl ester. Another equivalent of Grignard attacks again resulting in the tertiary alcohol. During this reaction tetrahydrofuran as the solvent and the ester can both chelate with the magnesium - this aids in the nucleophilic attack of the ester by polarising the carbonyl. In the presence of *N*-methylpyrrolidone (NMP) and tris(acetylacetonato)iron(III) ($Fe(acac)_3$) the chloride undergoes a substitution reaction with the Grignard reagent (Scheme 55).

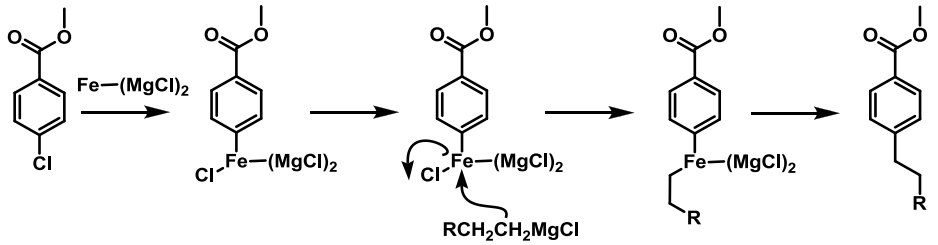


Scheme 55 Grignard reactions with and without NMP and $Fe(acac)_3$

NMP probably chelates to the magnesium and, due to conjugation from the lone pair on the nitrogen, this chelation is stronger than chelation by an ester. This has the affect of not polarising the ester carbonyl group thus not enhancing the likelihood of nucleophilic attack. $Fe(acac)_3$ is an Fe(III) complex and is only the pre-catalyst, the form in which iron is added to the reaction. The active species, believed to be an Fe(-II) complex, is formed from the reaction of five equivalents of Grignard reagent (Scheme 56) with $Fe(acac)_3$. The iron then inserts into the carbon-chlorine bond in an oxidative addition reaction. The alkyl anion attacks the iron, causing the chloride to leave, in a transmetallation reaction. Finally reductive elimination occurs, causing the loss of the iron to give the alkyl substituted product (Scheme 57).



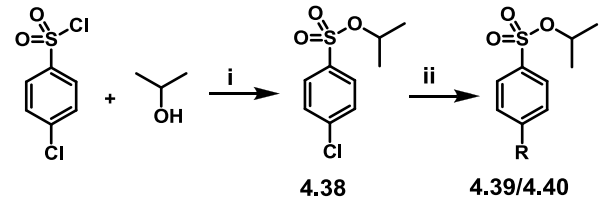
Scheme 56 Formation of $Fe(MgCl)_2$



Scheme 57 Reaction of $Fe(MgCl)_2$ with methyl-4-chlorobenzoate

The target compounds were 4-butylbenzenesulfonic acid and 4-hexylbenzenesulfonic acid. 4-Chlorobenzenesulfonylchloride was converted to the isopropylsulfonate ester (**4.38**), using propan-2-ol and DMAP as a nucleophilic

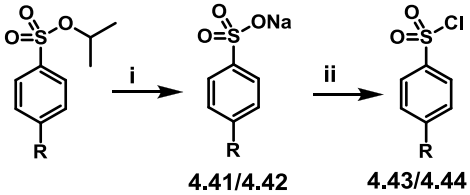
catalyst.[230] The iron cross-coupling reaction was then carried out to give compounds **4.39**-**4.40** (Scheme 58).



(4.38): (64 % yield), **(4.39):** R = Bu (57 % yield),**(4.40):** R = Hex (44 % yield)
Reagents; (i) CH_2Cl_2, 3 eq. DMAP, (ii) THF, 6 eq. NMP,
5 % $Fe(acac)_3$ and 1.25 eq. RMgCl

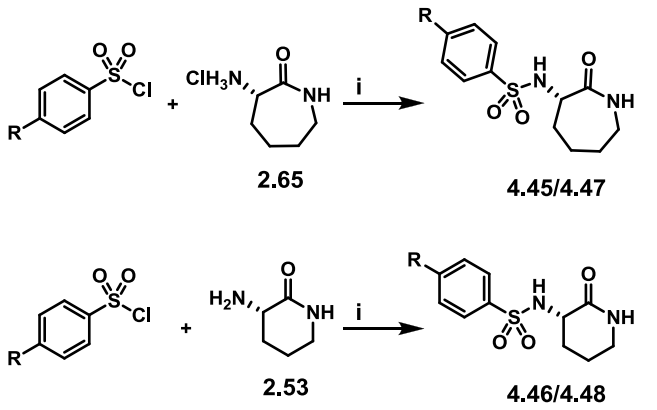
Scheme 58 Synthesis of compounds **4.38**-**4.40**

The sulfonate ester was removed using NaI (Scheme 59, **4.41**, **4.43**).[230] The sodium salt was converted into the sulfonyl chloride using the previously used method to give compounds (**4.43**) and (**4.44**) which were not isolated and coupled to the lactams under Schotten-Baumann conditions[185] (Scheme 60, **4.45**-**4.48**).



(4.41): R=Bu, **(4.42):** R=Hex, **(4.43):** R=Bu, **(4.44):** R=Hex
Reagents; (i) CH_3COCH_3, 5 eq. NaI,
(ii) CH_2Cl_2, $(COCl)_2$, DMF

Scheme 59 Synthesis of compounds **4.41**-**4.44**



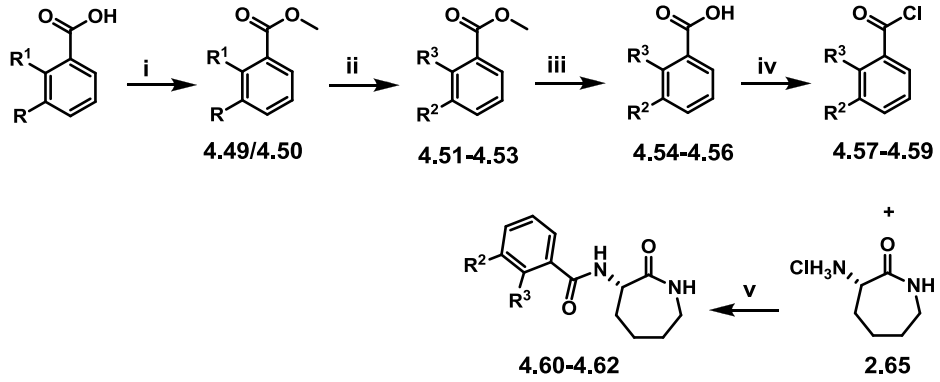
(4.45): R=nBu (19 % yield),**(4.46):** R=nBu (48 % yield),
(4.47): R=nHex (46 % yield), **(4.48):** R=nHex (17 % yield),
Reagents; (i) CH_2Cl_2/H_2O, 3 eq. Et_3N

Scheme 60 Synthesis of compounds **4.45**-**4.48**

4.4 3-Carboxy and 2-Carboxy Compounds

This reaction had previously been reported for 4-chloro substituted compounds but not so successfully for 3- or 2-chloro substituted compounds. The next target compounds were 3- or 2-alkyl substituted benzoyl lactams so methyl-3-chlorobenzoate (**4.49**) and methyl-2-chlorobenzoate (**4.50**) were synthesised from their corresponding acids using methanolic HCl (Scheme 61). The iron cross-coupling reaction was carried out as described above. Reaction times of 5 minutes, 15 minutes and 15 hours and all led to similar results. Some product was formed, (**4.51**-**4.53**) however some starting material remained. The unreacted starting material was inseparable from the product. After basic hydrolysis of the ester it was possible to separate the unreacted starting materials from the products. The starting material hydrolyses preferentially to the product so, on extraction, the hydrolysed starting materials remained in the aqueous layer allowing clean ester to be obtained. The clean ester was then subjected to another hydrolysis reaction to yield the acids (**4.54**-**4.56**). The acid chlorides, compounds (**4.57**-**4.59**), were synthesised as previously described and coupled to the lactam to give the following: 3-

butylbenzoyl-(*S*)-3-amino-azepan-2-one (**4.60**), 3-hexylbenzoyl-(*S*)-3-amino-azepan-2-one (**4.61**) and 2-butylbenzoyl-(*S*)-3-amino-azepan-2-one (**4.62**).

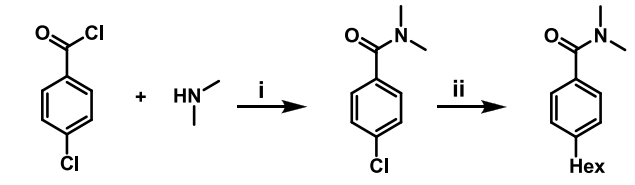


(4.49): R=Cl, R^1=H, (> 99 % yield), **(4.50):** R=H, R^1=Cl (> 99 % yield)
(4.60): R^2=nBu, R^3=H (6 % yield over 4 steps), **(4.61):** R^2=nHex, R^3=H (53 % yield over 4 steps),
(4.62): R^2=H, R^3=nBu, (2 % yield over 4 steps)
Reagents; (i) CH_3OH, 2 eq. AcCl, (ii) THF, 6 eq. NMP, 5 % $Fe(acac)_3$, RMgCl, (iii) H_2O, NaOH, (iv) CH_2Cl_2, $(COCl)_2$, DMF, (v) CH_2Cl_2/H_2O, 3 eq. Et_3N

Scheme 61 Synthesis of compounds **4.49**-**4.62**

4.5 Fe Cross-Coupling on Amides

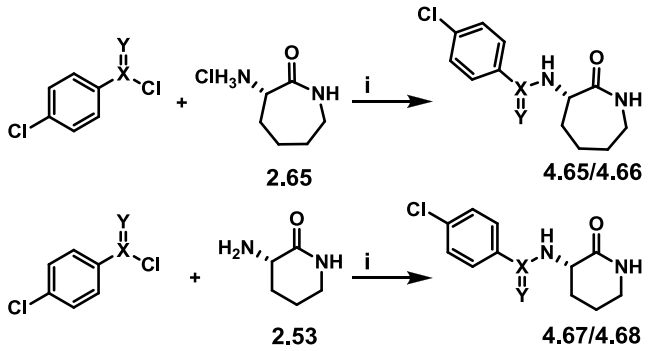
Because of the success of these reactions, the coupling was attempted with a tertiary amide instead of an ester. This involved the synthesis of 4-chlorobenzoyldimethylamide (**4.63**) from 4-chlorobenzoylchloride (Scheme 62). The iron cross-coupling reaction was carried under the same conditions, yielding the product, 4-hexylbenzoyldimethylamide (**4.64**), which was purified by flash column chromatography to remove excess NMP. The reaction was carried out using a *meta*-chloride, which as with the esters was not as clean although some product was formed. The reaction using an *ortho*-chloride was unsuccessful. Potential applications for this reaction would be to synthesise chlorobenzoyl lactams and perform iron cross coupling substitution reactions on these.



(4.63): (62 % yield), **(4.64):** (58 % yield)
Reagents; (i) CH_2Cl_2, 3 eq. Et_3N, (ii) THF, 6 eq. NMP, 5 % $Fe(acac)_3$, RMgCl

Scheme 62 Synthesis of compounds **4.63**-**4.64**

4-Chlorobenzoyl and 4-chlorobenzenesulfonyl lactams were synthesised from 4-chlorobenzoylchloride or 4-chlorobenzenesulfonylchloride and the 6-membered and 7-membered lactams **2.53** and **2.65** (Scheme 63). The resulting compounds **4.65**-**4.68** were all subjected to the iron cross-coupling reaction however no desired product was recovered.



(4.65): XY=CO (20 % yield),**(4.66):** XY=SO_2 (40 % yield),
(4.67): XY=CO (46 % yield), **(4.68):** XY=SO_2 (41 % yield),
Reagents; (i) CH_2Cl_2/H_2O, 3 eq. Et_3N

Scheme 63 Synthesis of compounds **4.65**-**4.68**

4.6 SSTR2 Binding Results

The SSTR2 binding data was determined by Glenda Chandler at Total Scientific (Babraham Research Campus, Cambridge) using FP by exactly the same method as for the compounds in Chapter Two. Data is given as percentage inhibition as SS-14 FITC from SSTR2. As expected, the compounds with the longer alkyl chains show

greater ability to displace somatostatin from binding to SSTR2. None of the compounds with an alkyl group in the *para* or *meta* position smaller than a hexyl group showed any activity. The results for the larger compounds (Figure 81) are shown in Table 12.

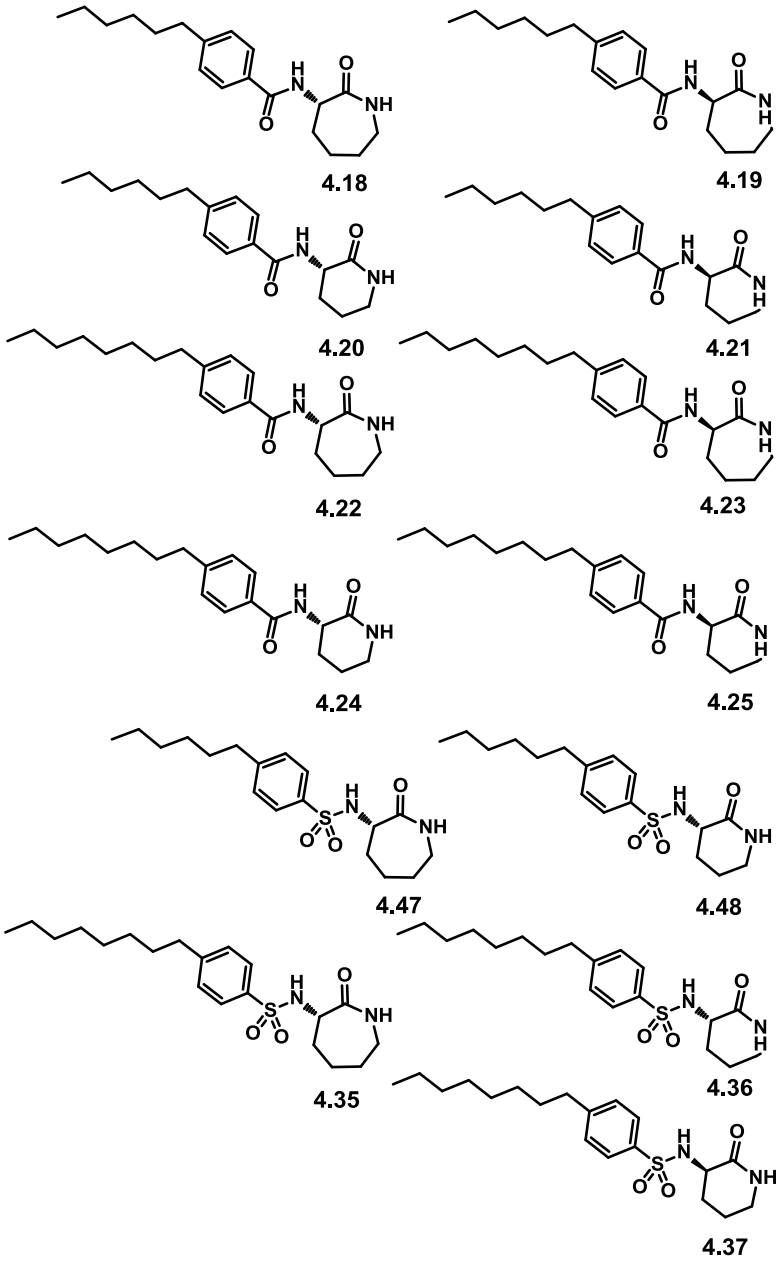


Figure 82 Compounds tested for SSTR2 binding

Compounds **4.36** and **4.37** showed the greatest activity at 100 % comparable to the SSTR2 ligands of Chapter 2. These were the 6-membered sulfonamides with the

octyl group substituent. The next best compound was **4.35**, the analogous 7-membered compound, but the activity has dropped down to 46 %. The hexyl sulfonamides **4.47** and **4.48** have similar activities of 28 and 27 % respectively, being the next most active compounds. From this data it can be concluded that the sulfonamides show greater activity than the carboxamides, with the most active being the octyl compounds. The 6-membered lactams are more active than the 7-membered, however the stereochemistry of the lactam does not seem to matter. This correlates with the previously synthesised alkyl sulfonamides of Chapter 3 in which the sulfonamide compound **3.17** showed greater activity of 58 % over the corresponding carboxamide compounds. The only other compounds that showed notable activity were **4.21** and **4.25**, the 6-membered hexyl and octyl carboxy compounds with (*R*)-stereochemistry. From this it can be concluded that again the 6-membered lactams are more active than the 7-membered lactam compounds. The stereochemistry does seem to have an effect as the corresponding (*S*)-compounds have lower activities. Although some strongly binding compounds have been synthesised the overall results seem varied and this may be explained by their solubility in the assay. The compounds showing lower than expected activity did not stay in solution when in 4 % aqueous DMSO. They were tested up to 10 % aqueous DMSO and still did not stay in solution, they could not be dissolved in any higher concentrations of DMSO as this would affect the assay. It has been shown that, in the case of the sulfonamides, high binding can be achieved by using the long chain and aromatic motifs on the lactam. It is possible that all the hexyl and octyl compounds would show high binding but are hindered by their poor solubility. As previously discussed the errors associated with the measurement in this assay are about 20 %.

Compound (7-membered)	% Somatostatin Inhibition	Compound (6-membered)	% Somatostatin Inhibition
4.18	11	**4.20**	9
4.19	10	**4.21**	25
4.22	1	**4.24**	-2
4.23	4	**4.25**	31
4.35	46	**4.36**	101
4.47	28	**4.37**	100
		4.48	27

Table 12 % inhibition of SS-14 FITC from SSTR2 (data from Glenda Chandler at Total Scientific)

Rough IC_{50} values were calculated for compounds **4.36** and **4.37** by performing a titration by the same method as those in Chapter 2. The values obtained were 50 and 49 μM for compounds **4.36** and **4.37** respectively. The values are 100-fold less potent than the compounds in Chapter 2, however they were not designed as SSTR2 ligands so this is not surprising.

4.7 Conclusions

These results indicate compounds with the longer chain substituents are required to displace somatostatin from binding. The substituent on the aromatic ring is required to be at least a hexyl group for this to occur. Not all the compounds with a hexyl or octyl substituent gave high inhibition values however this can possibly be attributed to their poor solubility as previously discussed. The unsubstituted 6-membered (**3.14**) and 7-membered (**3.28**) benzoylamino lactams from the data analysed at the start of Chapter 3 display inhibition values of 56 and 23 % respectively. It appears that these are better at displacing somatostatin than those with ethyl, butyl or tertiary butyl substituents which all have values of 0 % inhibition. It was the relatively high inhibition value of compound **3.14** compared to other BSCIs lacking long chain

substituents that prompted the synthesis of these alkyl benzoyl substituted lactams. It was thought that perhaps the presence of the aromatic ring enhanced the ability of the compound to inhibit the binding of somatostatin, and indeed does increase binding in relation to the unsubstituted compounds of Chapter 3. It has been shown that the ability to displace somatostatin from binding to SSTR2 does not necessarily translate into high BSCI activity. Leukocyte migration data is available for the non-substituted benzoyl lactams, compounds **3.14** and **3.28** of 400 and 90 pM respectively, the methyl substituted benzoyl lactams, compounds **4.69** and **4.70** of 60 and 90 pM respectively, and of the tosyl substituted lactams, compounds **4.71** and **4.72** of 10 and 200 pM respectively (Figure 83).[202] These show high levels of leukocyte migration inhibition, therefore for comparison leukocyte migration data is required for the compounds synthesised herein. However the insolubility of these compounds as shown in particular by the hexyl and octyl compounds may pose a problem in the leukocyte migration assay and suggest these compounds would not be suitable drug candidates.

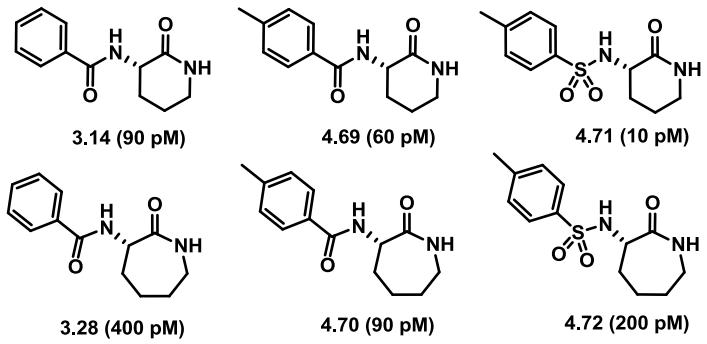


Figure 83 Compounds **3.14, 3.28** and **4.69-4.72** (leukocyte migration inhibition shown in brackets, data from Dr Jill Reckless, University of Cambridge)

4.8 References

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Chapter 5 - Conclusions

The results obtained indicate a split binding site model for SSTR2. It has been concluded that BSCIs exert their action through SSTR2 because a large number of them have been shown to displace somatostatin, the endogenous ligand, from binding.[51] Likewise a large number of BSCIs do not displace somatostatin but strongly inhibit leukocyte migration.[46-48] It is therefore evident that to be good BSCIs the compounds do not need to displace somatostatin from its binding site on SSTR2,

and therefore they may not bind in exactly the same binding site as somatostatin. BSCIs with long chains substituted at the 2-position are more potent competitive ligands for SSTR2. BSCIs with long unsubstituted chains do not displace somatostatin which means the side chains of certain compounds are in such a conformation that they do not block somatostatin binding. From this it can be concluded that the binding site for the lactam is close and perhaps partially overlapping to the binding site of somatostatin. In a similar fashion BSCIs lacking a large side chain are too able to bind without blocking somatostatin. The compounds in Chapter 2 displayed a correlation between high somatostatin displacement and high BSCI activity. These were the compounds structurally based on known SSTR2 ligands therefore high SSTR2 binding was expected, evidently the tight binding of those compounds allowed the BSCI activity to be stimulated also.

It was initially thought that, like somatostatin, the KWF mimic ligands bound to the orthosteric binding site of SSTR2 resulting in affects such as cAMP and growth hormone regulation (Figure 84). Somatostatin shows only approximately 50 % maximal effect in the leukocyte migration inhibition assay. It is was expected the KWF mimics would also show low BSCI activity. It was expected likewise that BSCIs bound to an adjacent allosteric site have the potential to block the orthosteric site preventing somatostatin from binding.

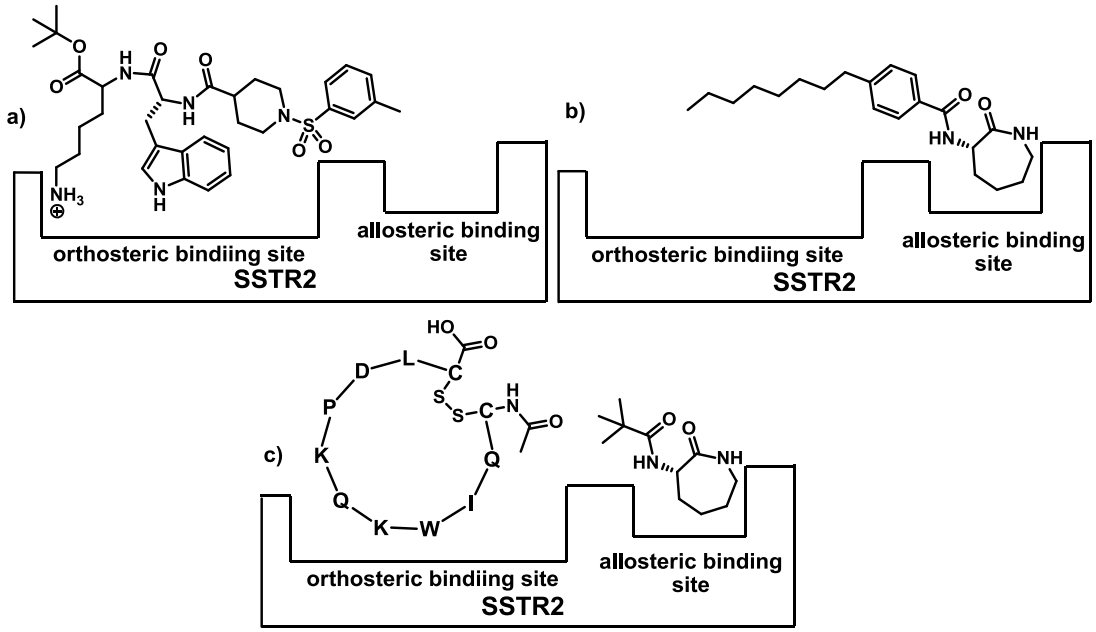


Figure 84 The predicted binding patterns different ligands at SSTR2, a) KWF ligands bind at an orthosteric site, b) lactams at an adjacent allosteric site and either displace somatostatin, or c) bind alongside somatostatin.

This study has revealed that an anti-inflammatory affect can be exerted through both the orthosteric and allosteric binding site. The KWF mimic compound **2.51** binds with a similar affinity to SSTR2 as somatostatin, however like the other KWF mimics is a much more potent BSCI, which is exerted though the orthosteric site. Somatostatin is a weak BSCI. Therefore it is concluded that although the binding patterns seems to be the same as shown in Figure 84 certain ligands are able to exert an anti-inflammatory response through the orthosteric site. SSTR2 is functionally selectivity as the KWF mimics, somatostatin and acylaminolactams are producing different cellular responses. The allosteric part of the binding site is the area in which the lactams bind which again causes an anti-inflammatory response, which, in the case of a number of molecules, is much more potent than somatostatin (Figure 85).

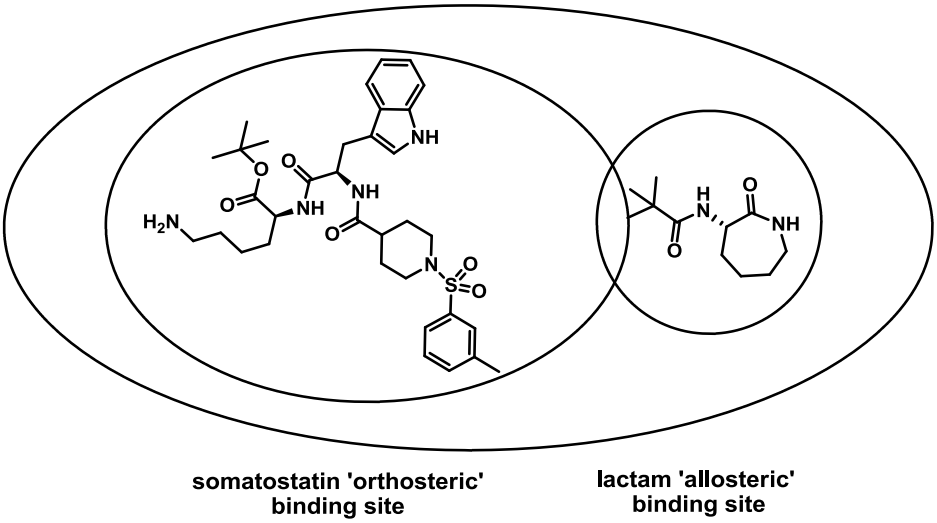


Figure 85 Split binding site model for SSTR2

Intracellular effects are caused by the protein-protein interactions of the receptor-ligand complex and intracellular proteins. Different ligands result in different receptor-ligand conformations, thus different intracellular protein-protein interactions therefore different intracellular responses. It is possible that the anti-inflammatory affect exerted by small acylaminolactams and KWF mimics require a more sterically crowded receptor-ligand complex. The much larger size of somatostatin may preclude this "BSCI" conformation inhibiting full anti-inflammatory activity.

The most important aspect regarding the future work of BSCIs would be to prove that they bind to SSTR2 by the synthesis of a fluorescently or radio labelled ligand. This would enable information to be directly gained on the binding affinity of the compounds and information on inhibitors, as well as giving a clearer picture of the mechanism of which they act through. Due to the selection of a clinical candidate FX125L which has performed well on Phase I clinical trials, new small molecules are not required for drug development. The compounds synthesised herein have

shown similar BSCI activity to the current clinical candidate and other factors such as metabolic stability and ease of synthesis have supported its choice.

Chapter 6 - Experimental

6.1 General Experimental

All experiments were carried out in fume hoods, adhering to general laboratory safety protocols and risk assessments (COSHH). Reagents and chemicals were purchased from Sigma Aldrich, Lancaster, TCI, Alfa Aesar or Bachem and used without further purification. *p*H 2 buffer was made with a 0.75 M solution of $NaSO_4$ and a 0.25 M solution of H_2SO_4.

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker Avance DRX 400 MHz fourier transform machine at room temperature (298 K) unless otherwise stated. Chemical shifts are quoted in parts per million downfield of tetramethylsilane. Solvents were used as an internal standard when assigning NMR spectra (δ_H: $CDCl_3$ 7.26 ppm, CD_3OD 3.31 ppm, d^6-DMSO 2.50 ppm, D_2O 4.79 ppm; δ_C: $CDCl_3$ 77.1 ppm, CD_3OD 49.0 ppm, d^6-DMSO 39.5 ppm. Coupling constants, *J*, are given in Hz to the nearest 0.5. ^{13}C-NMR spectra were recorded with broadband proton decoupling and spectra assigned on the basis of pendant, 3D COSY and HMQC spectra. Infra-red spectra were recorded on a Avatar 320. $[\alpha]_D$ values were recorded on an optical activity AA 1000 polarimeter set at 598 nm (sodium D line) and units are 10^{-1} deg cm^2 g^{-1}. The samples were made to C = 1 ≡ 10 mg/mL using spectroscopic grade MeOH or chloroform. ESI mass spectra were obtained on a Bruker Esquire 2000 mass spectrometer coupled with an Agilent 1100 HPLC (without a column) as the delivery system. Accurate mass spectra were obtained using a Bruker micro-TOF ESI attached to a time of flight (TOF) analyser, CHN elemental analyses were carried out by Warwick Analytical Services. Thin Layer Chromatography (TLC) was performed using silica (0.25 mm) coated alumina

plates. Melting points were determined using a Stuart SMP10 melting point machine. The optical purity was measured by HPLC analysis using a Varian Prostar 335 Photodiode Array Detector, using a Varian Prostar Solvent Delivery Module and a Varian Prostar 420 Autosampler. The *e.e*.s were determined using a CHIRALPAK AS column (4.6 mm × 250 mm) (*n*-hexane/propan-2-ol = 1:1; flow rate, 0.5 mL/min, λ = 207 nm).

6.2 Chapter 2 Experimental

6.2.1 Potential SSTR2 ligands - Non-Lactam Containing Compounds

2.10, *N-meta*-Toluenesulfonyl isonipecotic acid

Isonipecotic acid (1.30 g, 10.1 mmol) was dissolved in H_2O (30 mL) and THF (30 mL). *meta*-Toluenesulfonylchloride (1.45 mL, 10.0 mmol) and triethylamine (4.2 mL, 30 mmol) were added and the reaction was stirred on ice overnight. The organic solvents were removed *in vacuo* and the reaction acidified with 4M H_2SO_4 to pH 2. The product was extracted with ethyl acetate, dried over Na_2CO_3 and reduced *in vacuo.* The product was purified by recrystallisation from hot ethyl acetate to give compound **2.10** as a white solid (2.16 g, 76 %); *mp* 146-147 °C; υ_{max}/cm^{-1}: 2882 (saturated C-H), 1697 (C=O) and 1333, 1151 (SO_2); *Anal.* ($C_{13}H_{17}NO_4S$ requires: C; 55.11, H; 6.05, N; 4.94) found: C; 54.39, H; 6.05, N; 4.94; δ_H (300 MHz, $CDCl_3$) 10.30 (br.s, 1H, O*H*), 7.55-7.51 (m, 2H, C*H*-CH-CCH$_3$ and C*H*-C-CH$_3$,), 7.40-7.38 (m, 2H, C*H*-CSO$_2$), 3.65 (td, 2H, *J* 12, 3.5, equatorial C*H*$_2$-N), 2.43 (qd, 2H, *J* 11.5, 3, axial C*H*$_2$-N), 2.42 (s, 3H, C*H*$_3$), 2.27 (tt, 1H, *J* 10.5, 4, C*H*-COOH), 1.98 (dd, 2H, *J* 13, 3.5, equatorial C*H*$_2$-CH$_2$N) and 1.80 (qd, 2H, *J* 12, 4, axial C*H*$_2$-CH$_2$-N); δ_C (75 MHz, $CDCl_3$) 179.3 (*C*=O), 138.7 (*C*-CH$_3$), 135.2 (*C*-SO$_2$), 133.1 (C*H*-C-CH$_3$), 128.3 (C*H*-CHCCH$_3$), 127.3 (*C*H-SO$_2$), 124.2 (*C*H-CHCHCCH$_3$), 45.8 (*C*H$_2$-N),

39.2 (<u>C</u>H-COOH), 26.6 (<u>C</u>H_2-CH_2N) and 20.8 (<u>C</u>H_3); ESI *m/*z 100 %, 284.1 (MH^+) and 24 %, 306.2 (MNa^+).

2.11, *N-para*-Toluenesulfonyl isonipecotic acid

Isonipecotic acid (0.71 g, 5.5 mmol) was dissolved in H_2O (25 mL) and THF (25 mL). *para*-Toluenesulfonylchloride (0.99 g, 5.2 mmol) and triethylamine (2.1 mL, 15 mmol) were added and the reaction was stirred on ice overnight. The THF was removed *in vacuo* and the reaction acidified with 4M H_2SO_4 to pH 2. The product was extracted with ethyl acetate, dried over Na_2CO_3 and reduced *in vacuo* to give compound 2.11 as a white solid (1.19 g, 84 %); *mp* 165-167 ºC (Lit. 169-170 °); υ_{max}/cm^{-1}: 2871 (saturated C-H) 1696 (C=O) and 1331, 1157 (SO_2); *Anal.* ($C_{13}H_{17}NO_4S$ requires: C; 55.11, H; 6.05, N; 4.94) found: C; 55.12, H; 6.04, N; 5.02; δ_H (400 MHz, $CDCl_3$) 7.63 (d, 2H, *J* 8, C<u>*H*</u>-CSO_2), 7.32 (d, 2H, *J* 8, C<u>*H*</u>-CCH_3), 3.64 (dt, 2H, *J* 12, 3, equatorial C<u>*H*</u>$_2$-N), 2.05 (s, 3H, CH_3), 2.14-2.01 (m, 2H, axial C<u>*H*</u>$_2$-N), 1.89 (tt, 1H, *J* 4, 2.5, C<u>*H*</u>-COOH), 1.56 (dq, 2H, *J* 12, 3, equatorial C<u>*H*</u>$_2$-CH-COOH) and 1.42 (qd, 2H, *J* 11, 4, axial C<u>*H*</u>$_2$-CH-COOH); δ_C (100 MHz, $CDCl_3$) 180.2 (<u>C</u>=O), 143.9 (<u>C</u>-CH_3), 133.3(<u>C</u>-SO_2), 129.9 (<u>C</u>H-CCH_3), 127.9 (<u>C</u>H-CSO_2), 45.6 (<u>C</u>H_2-N), 40.1 (<u>C</u>H-COOH), 27.5 (<u>C</u>H_2-CH-COOH) and 21.8 (<u>C</u>H_3); HR ESI *m/*z ($C_{13}H_{17}NO_4SNa^+$ requires 306.0770) found 306.0761. Data is consistent with previously reported data for this compound.[231]

2.12, *N*-Benzoyl isonipecotic acid

Isonipecotic acid (1.29 g, 9.99 mmol) was dissolved in H_2O (50 mL) and THF (50 mL). Benzoyl chloride (1.2 mL, 10 mmol) and triethylamine (4.2 mL, 30 mmol) were added and the reaction was stirred on ice overnight. The THF was removed *in vacuo* and the reaction acidified with 4M H_2SO_4 to pH 2. The product was extracted

with ethyl acetate, and dried over Na_2SO_4 and reduced *in vacuo* to give compound 2.12 as a white solid (1.57 g, 67 %); *mp* 129-130 ºC; υ_{max}/cm^{-1}: 2923 (saturated C-H), 2858 (O-H), 1730 (acid C=O) and 1612 (amide C=O); *Anal.* (Calcd for $C_{13}H_{15}NO_3.1/17\ H_2O$: C, 66.63; H, 6.50; N, 5.98) Found: C, 66.61; H, 6.50; N, 5.90; δ_H (400 MHz, $CDCl_3$) 11.06 (s, 1H, O*H*), 7.28-7.16 (m, 5H, phenyl), 4.48 (br.s, 1H, equatorial C*H*$_2$-NCO), 3.71 (br.s, 1H, equatorial C*H*$_2$-NCO), 3.16 (br.t, 2H, axial C*H*$_2$-NCO), 2.58 (tt, 1H, *J* 4, 2.5, C*H*-COOH) and 1.88-1.37 (m, 4H, axial and equatorial CH$_2$-CH-COOH); δ_C (100 MHz, $CDCl_3$) 178.5 (*C*=O acid), 170.9 (*C*=O-amide), 135.5 (*i*-phenyl *C*H), 129.8 (*p*-phenyl *C*H), 128.5 (*m*-phenyl *C*H), 126.9 (*o*-phenyl), 47.6 (*C*H$_2$-NCO), 41.6 (*C*H$_2$-NCO), 40.6 (*C*H-COOH), 28.5 (*C*H$_2$-CH-COOH) and 27.5 (*C*H$_2$-CH-COOH); HR ESI *m/*z ($C_{13}H_{15}NO_3Na^+$ requires 256.0944) found 256.0946. Data is consistent with previously reported data for this compound.[232]

2.04, *N*-(2-(1*H*-Indol-3-yl)ethyl)-1-(*meta*-tolylsulfonyl)piperidine-4-carboxamide

Acid **2.10** (1.81 g, 3.39 mmol) was dissolved in dichloromethane (40 mL) and cooled to 0° C. HATU (2.53 g, 6.65 mmol) was added and the mixture was stirred for 10 minutes. Tryptamine (1.05 g, 6.55 mmol) and triethylamine (2.7 mL, 19 mmol) were added and the reaction was stirred over night. The dichloromethane was removed *in vacuo*, the product was dissolved in ethyl acetate and washed with a pH 2 buffer (3 × 10 mL) and ½ saturated $NaHCO_3$ (3 × 10 mL). The organic layer was dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate:MeOH 50:50:0 to 0:70:30) to give compound **2.04** as a white solid (0.87 g, 32 %); *mp* 142-143 º C; υ_{max}/cm^{-1}: 3390 (N-H), 2924 (saturated C-H), 1655 (C=O amide), 1556 (aromatic) and 1330, 1156

(SO_2); δ_H (400 MHz, $CDCl_3$) 8.19 (br.s, 1H, indole N*H*), 7.57-7.50 (m, 3H, indole C*H*-C-C, C*H*-CSO_2 and C*H*-$CHCHCCH_3$), 7.40-7.34 (m, 3H, indole C*H*-C-NH, C*H*-$CHCCH_3$ and C*H*-CCH_3), 7.19 (t, 1H, *J* 7.5, indole C*H*-CH-C), 7.09 (t, 1H, *J* 7.5, indole C*H*-CH-C-C), 6.97 (d, 1H, *J* 2, indole C*H*-NH), 5.54 (t, 1H, *J* 5.5, N*H*-CH_2), 3.71 (dt, 2H, *J* 12, 3.5, equatorial C*H*$_2$-N), 3.55 (q, 2H, *J* 6.5, C*H*$_2$-CH_2-indole), 2.93 (t, 2H, *J* 6.5, C*H*$_2$-indole), 2.41 (s, 3H, C*H*$_3$), 2.30 (td, 2H, *J* 11.5, 3.5, axial C*H*$_2$-N), 1.91 (tt, 1H, *J* 11.5, 4, C*H*-CO) and 1.81 (m, 4H, C*H*$_2$-CH_2N); δ_C (100 MHz, $CDCl_3$) 173.9 (*C*=O), 139.4 (*C*-CH_3), 136.5 (indole *C*-NH), 135.9 (*C*-SO_2), 133.72 (*C*H-CCH_3), 129.0 (*C*H-$CHCCH_3$), 127.9 (*C*H-$CHCHCCH_3$), 127.3 (indole *C*-CNH), 124.8 (*C*H-CSO_2), 122.2 (indole *C*H-NH), 122.1 (indole *C*H-CH-C-C), 119.4 (indole *C*H-CH-C), 118.6 (indole *C*H-C-C), 112.6 (indole C-CH_2), 111.4 (indole *C*H-C-NH), 45.6 (*C*H$_2$-N), 42.0 (*C*H-CONH), 39.8 (*C*H$_2$-NH), 28.1 (*C*H$_2$-CH_2N), 25.1 (*C*H$_2$-indole), and 21.4 (*C*H$_3$); ESI *m/*z 100 %, 448.1 (MNa^+).

2.13, *N*-(2-(1*H*-Indol-3-yl)ethyl)-1-tosylpiperidine-4-carboxamide

Acid **2.11** (0.60 g, 2.1 mmol) was dissolved in dichloromethane (20 mL) and cooled to 0° C. HATU (0.76 g, 2.0 mmol) was added and the mixture was stirred for 10 minutes. Tryptamine (0.33 g, 2.1 mmol) and triethylamine (0.8 mL, 6 mmol) were added and the reaction was stirred over night. The dichloromethane was removed *in vacuo*, the product was dissolved in ethyl acetate and washed with a pH 2 buffer (3 × 10 mL) and ½ saturated $NaHCO_3$ (3 × 10 mL). The organic layer was dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate:MeOH 50:50:0 to 0:70:30) to give compound **2.13** as a white solid (0.42 g, 48 %); *mp* 186-188 ° C; υ_{max}/cm^{-1}: 3384 (N-H), 2928 (saturated C-H), 1633 (C=O amide), 1559 (aromatic), and 1329, 1160

(SO_2); δ_H (400 MHz, d^6-DMSO) 10.77 (s, 1H, N*H* indole), 7.80 (t, 1H, *J* 6, N*H*-CH_2), 7.62 (d, 2H, *J* 8, C*H*-C-SO_2), 7.50 (d, 1H, *J* 7.5, indole C*H*-C-C), 7.44 (d, 2H, *J* 8, C*H*-C-CH_3), 7.32 (d, 1H, *J* 8, indole C*H*-C-NH), 7.10 (d, 1H, *J* 2, indole C*H*-NH), 7.05 (t, 1H, *J* 7.5, indole C*H*-CH-C), 6.96 (t, 1H, *J* 7.5, indole C*H*-CH-C-C), 3.56 (br.d, 2H, *J* 12, equatorial CH_2-N), 3.28 (q, 2H, *J* 7, CH_2-CH_2-indole), 2.77 (t, 2H, *J* 7.5, CH_2-indole), 2.40 (s, 3H, CH_3), 2.24 (td, 2H, *J* 11.5, 2.5, axial CH_2-N), 2.05 (tt, 1H, *J* 11.5, 4, C*H*-CCO), 1.72 (dd, 2H, *J* 14, 3, equatorial CH_2-CH_2N), and 1.55 (qd, 2H, *J* 12.5, 4, axial CH_2-CH_2N); δ_C (100 MHz, d^6-DMSO) 173.3 (*C*=O), 143.4 (*C*-CH_3), 136.2 (*C*-NH), 132.6 (*C*-SO_2), 129.8 (*C*H-C-CH_3), 127.4 (*C*H-C-SO_2), 127.2 (indole *C*-C-NH), 122.6 (*C*H-NH), 120.9 (*C*H-CH-C-C), 118.2 (*C*H-C-C), 118.2 (*C*H-CH-C), 111.7 (*C*-CH_2), 111.3 (*C*H-C-NH), 45.3 (*C*H_2-N), 40.4 (*C*H-CONH), 39.4 (*C*H_2-NH), 27.7 (*C*H_2-CH_2N), 25.1 (*C*H_2-indole) and 21.5 (*C*H_3); ESI *m/*z 100 %, 448.2 (MNa^+) and 5 %, 426.2 (MH^+); HR ESI *m/*z ($C_{23}H_{27}N_3O_3SNa^+$ requires 448.1665) found 448.1677.

2.14, *N*-(2-(1*H*-Indol-3-yl)ethyl)-1-benzoylpiperidine-4-carboxamide

Acid **2.12** (0.93 g, 3.4 mmol) was dissolved in dichloromethane (30 mL) and cooled to 0° C. HATU (1.57 g, 4.13 mmol) was added and the mixture was stirred for 30 minutes. Tryptamine (0.64 g, 3.4 mmol) and triethylamine (1.7 mL, 12 mmol) were added and the reaction was stirred for 48 hours. The dichloromethane was removed *in vacuo*, the product was dissolved in ethyl acetate and washed with a pH 2 buffer (3 × 10 mL) and ½ saturated $NaHCO_3$ (3 × 10 mL). The organic layer was dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (ethyl acetate:MeOH 100:0 to 70:30) to give compound **2.14** as a white solid (0.72 g, 48 %); *mp* 183-185 °C; υ_{max}/cm^{-1}: 3278 (N-H), 2919 (saturated

C-H), 1652 (C=O amide) and 1599 (aromatic); δ_H (400 MHz, d^6-DMSO) 10.42 (s, 1H, N*H* indole), 7.54 (d, 1H, *J* 8, indole C*H*-C-C), 7.45-7.41 (m, 3H, phenyl *m*-C*H* and *p*-C*H*), 7.38-7.33 (m, 3H, indole C*H*-C-NH and phenyl *o*-C*H*), 7.09 (s, 1H, indole C*H*-NH), 7.06 (t, 1H, *J* 8, indole C*H*-CH-C), 6.98 (t, 1H, *J* 7.5, indole C*H*-CH-C-C), 3.99 (br.d, 2H, *J* 11, equatorial CH_2-N), 3.41 (q, 2H, *J* 6.5, CH_2-NH), 2.98 (td, 2H, *J* 12, 3, axial CH_2-N), 2.88 (t, 2H, *J* 7, CH_2-indole), 2.41 (tt, 1H, *J* 13.5, 4, C*H*-CO), 1.73 (dd, 2H, *J* 13, 3, equatorial CH_2-CH_2N) and 1.57 (qd, 2H, *J* 11.5, 4, axial CH_2-CH_2N); δ_C (100 MHz, *d*-DMSO) 173.6, 169.0 (*C*=O), 136.3 & 136.2 (C6 & C12), 129.3 (C11), 128.4 (C10), 127.3 (C7), 126.6 (C9), 122.6 (C1), 120.8 (C4), 118.2 (C5), 118.16 (C3), 111.8 (C8), 111.3 (C2), 41.8 (*C*H pip), 40.1 (*C*H_2-N), 39.1 (*C*H_2-NH), 30.0, 28.3 (*C*H_2-CH_2-N) and 25.2 (CH_2-indole); ESI *m/*z 100 %, 398.2 (MNa^+) and 9 %, 376.3 MH^+); HR ESI *m/*z ($C_{23}H_{25}N_3O_2Na^+$ requires 398.1839) found 398.1836.

2.15, *N*-(2-(1*H*-Indol-3-yl)ethyl)biphenyl-4-carboxamide

4-Biphenyl carboxylic acid (0.93 g, 4.7 mmol) was dissolved in dichloromethane (40 mL) and cooled to 0° C. HATU (1.83 g, 4.81 mmol) was added and the mixture was stirred for 4 hours. Tryptamine (0.68 g, 4.2 mmol) and triethylamine (2 mL, 14 mmol) were added and the reaction was stirred for 48 hours. The dichloromethane was removed *in vacuo*, the product was dissolved in ethyl acetate and washed with a pH 2 buffer (3 × 20 mL) and ½ saturated $NaHCO_3$ (3 × 20 mL). The organic layer was dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate:MeOH 50:50:0 to 0:70:30) to give compound **2.15** as a beige solid (0.88 g, 55 %); *mp* 171-173 °C; υ_{max}/cm^{-1}: 3422 (N-H), 1618 (C=O amide) and 1538 (aromatic); δ_H (400 MHz, d^6-DMSO) 10.82 (s,

1H, N*H* indole), 8.67 (t, 1H, *J* 6, N*H*-CH_2), 7.97 (d, 2H, *J* 8.5, biphenyl C*H*-C-CO), 7.76 (d, 2H, *J* 8.5, biphenyl C*H*-C-phenyl), 7.73 (d, 2H, *J* 8, biphenyl C*H*-C-aryl), 7.61 (d, 1H, *J* 8, indole C*H*-C-C), 7.49 (t, 1H, *J* 8, biphenyl C*H*-CH-C-aryl), 7.40 (t, 1H, *J* 7.5, biphenyl C*H*-CH-CH-C-aryl), 7.36 (d, 1H, *J* 8, indole C*H*-C-NH), 7.20 (d, 1H, *J* 2, indole C*H*-NH), 7.08 (t, 1H, *J* 7.5, indole C*H*-CH-C), 6.99 (t, 1H, *J* 7.5, indole C*H*-CH-C-C), 3.59 (q, 2H, *J* 7, C*H*$_2$-CH_2-indole) and 2.99 (t, 2H, *J* 7.5, C*H*$_2$-indole); δ_C (100 MHz, *d*-DMSO) 165.8 (*C*=O), 142.6, 139.2 (biphenyl *C*-aryl), 136.3 (indole *C*-NH), 133.5 (biphenyl *C*-CO), 129.0 (biphenyl *C*H-CH-C-aryl), 128.0 (biphenyl *C*H-C-CO), 127.8 (biphenyl *C*H-C-phenyl), 127.3 (indole *C*-C-NH), 126.8 (biphenyl *C*H-C-aryl), 126.4 (biphenyl *C*H-CH-CH-C-aryl), 122.6 (indole *C*H-NH), 120.9 (indole *C*H-CH-C-C), 118.3 (indole *C*H-C-C), 118.2 (indole *C*H-CH-C), 111.9 (indole *C*-CH_2), 111.4 (indole *C*H-C-NH), 40.2 (*C*H_2-NH), 25.2 (*C*H_2-indole); ESI *m/*z 100 %, 340.3 (MH^+) and 17 %, 363.1 (MNa^+); HR ESI *m/*z ($C_{23}H_{20}N_2ONa^+$ requires 363.1468) found 363.1465. NMR data is consistent with previously reported data for this compound.[233]

2.16, Boc-D-tryptophan

D-Tryptophan (2.04 g, 9.99 mmol) was dissolved in THF (20 mL), H_2O (20 mL) and cooled to 0 ºC. Di-*tert*-butyl-dicarbonate (2.18 g, 9.99 mmol) and triethylamine (2.8 mL, 20 mmol) were added and the reaction was stirred for 12 hours. The THF was removed *in vacuo*; ethyl acetate (30 mL) was added and the organic layer was separated and washed with pH 2 buffer (3 × 25 mL). The organic layer was then dried over Na_2SO_4 and reduced *in vacuo*, purified by re-crystallisation with ethyl acetate and petroleum ether to give compound **2.16** as a white solid (0.39 g, 13 %); *mp* 133-135 ºC (Lit. 135-136 °); $[\alpha]^{25}_D$ (c = 1.08, acetic acid) + 19.12 (Lit. -18.2 for

L enantiomer); υ_{max}/cm^{-1}: 3373 (N-H), 2979 (O-H), 1719, 1702 (C=O), 1702 and 1246 (C-O); δ_H (400 MHz, d_6-DMSO) 12.53 (1H, br.s, O*H*), 10.82 (s, 1H, N*H* indole), 7.50 (d, 1H, *J* 8, indole C*H*-C-C), 7.34 (d, 1H, *J* 8, indole C*H*-C-NH), 7.15 (d, 1H, *J* 4, N*H*-COO), 7.06 (t, 1H, *J* 7.5, indole C*H*-CH-C-NH), 6.98 (t, 1H, *J* 7.5, indole C*H*-CH-C-C), 6.95 (s, 1H, indole C*H*-NH), 4.15 (td, 1H, *J* 9, 4, C*H*-CH_2-indole), 3.13 (dd, 1H, *J* 14, 4 CH_2-indole), 2.98 (dd, 1H, *J* 14, 10, CH_2-indole) and 1.33 (s, 9H, C(CH_3)$_3$); δ_C (100 MHz, d_6-DMSO) 173.9 (*C*=O acid), 155.4 (*C*=O carbamate), 136.0 (indole *C*-NH), 127.2 (indole *C*-C-NH), 123.6 (indole *C*H-NH), 120.9 (indole *C*H-CH-C), 118.3 (indole *C*H-C-C), 118.1 (indole *C*H-CH-C-C), 111.4 (indole *C*H-C-NH), 110.2 (indole *C*-CH_2), 78.0 (*C*(CH_3)$_3$), 54.5 (*C*H-CH_2-indole), 28.2 (C(*C*H_3)$_3$) and 26.8(*C*H_2-indole); ESI *m/*z 100 %, 327.1 (MNa^+). Data is consistent with previously reported data for this compound.[234]

2.22, (*R*)-*tert*-Butyl-3-(1*H*-indol-3-yl)-1-oxo-1-(propylamino)propan-2-ylcarbamate

Acid **2.16** (3.40 g, 11.2 mmol) was dissolved in dichloromethane (100 mL) and cooled to 0°C. HATU (4.10 g, 10.8 mmol) was added and the mixture was stirred for 4 hours. Proplyamine (0.90 mL, 11 mmol) and triethylamine (4.70 mL, 33.6 mmol) were added and the reaction was stirred over night. The dichloromethane was removed *in vacuo*, the product dissolved in ethyl acetate and washed with a pH 2 buffer (3 × 25 mL), 0.1 M HCl (3 × 25 mL) and ½ saturated $NaHCO_3$ (3 × 25 mL). The organic solvents were removed *in vacuo*. The product was purified by recrystallisation from ethyl acetate and petroleum ether to give compound **2.22** as a white solid (3.04 g, 79 %); *mp* 133-135 º C; $[\alpha]^{29}_D$ (c = 0.256, MeOH) -2.73; υ_{max}/cm^{-1}: 3329 (N-H), 2934 (saturated C-H), 1687, 1646 (C=O), and 1246 (C-O); *Anal.* Calcd for $C_{19}H_{27}N_3O_3$: C, 66.06; H, 7.88; N, 12.16. Found: C, 65.80; H, 7.88; N,

12.07; δ_H (600 MHz, $CDCl_3$) 8.34 (s, 1H, indole NH), 7.66 (d, 1H, *J* 8, indole C*H*-C-C), 7.35 (d, 1H, *J* 8.5, indole C*H*-C-NH), 7.19 (t, 1H, *J* 8, indole C*H*-CH-C), 7.12 (t, 1H, *J* 7.5, indole C*H*-CH-C-C), 7.02 (s, 1H, indole C*H*-NH), 5.75 (s, 1H, N*H*-$CH_2CH_2CH_3$), 5.22 (s, 1H, N*H*-COO), 4.40 (br.s, 1H, C*H*-CH_2-indole), 3.30 (br.d, 1H, *J* 12, C*H*$_2$-indole), 3.19-2.99 (m, 3H, C*H*$_2$-indole and C*H*$_2$-CH_2-CH_3), 1.42 (s, 9H, C(C*H*$_3$)$_3$), 1.34-1.22 (m, 2H, C*H*$_2$-CH_3) and 0.72 (br.t, 3H, *J* 6.5, C*H*$_3$-CH_2); δ_C (150 MHz, $CDCl_3$) 171.6 (*C*=O amide), 155.5 (*C*=O carbamate), 136.4 (indole *C*-NH), 127.4 (indole *C*-C-NH), 123.2 (indole *C*H-NH), 122.5 (indole *C*H-CH-C-C), 119.7 (indole *C*H-C-C), 118.9 (indole *C*H-CH-C), 111.2 (indole *C*H-C-NH), 110.8 (indole *C*-CH_2), 80.0 (*C*(CH_3)), 55.3 (*C*H-CH_2-indole), 41.2 (*C*H_2-NHCO), 30.9 (*C*H_2-indole), 29.7 (C(*C*H_3)) 22.5 (*C*H_2-CH_3) and 11.1 (*C*H_3-CH_2); ESI *m/*z 100 %, 368.1 (MNa^+).

2.23, (*R*)-*O*-Benzyl *N*-(4-(2-*tert*butylcarbonylamino)-3-(1*H*-indol-3-yl)propanamido)butylcarbamate

Acid **2.16** (0.57 g, 1.8 mmol) was dissolved in dichloromethane (20 mL) and cooled to 0°C. HATU (0.82 g, 2.2 mmol) was added and the mixture was stirred for 10 minutes. *N*-Z,1,4-Diaminobutane.HCl (0.51 g, 1.9 mmol) and triethylamine (0.80 mL, 5.7 mmol) were added and the reaction was stirred over night. The dichloromethane was removed *in vacuo*, the product dissolved in ethyl acetate and washed with a pH 2 buffer (3 × 15 mL) and ½ saturated $NaHCO_3$ (3 × 15 mL). The organic layer was dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate, 70:30 to 50:50) to give compound **2.23** as a white solid (0.65 g, 67 %); *mp* 134-135 º C; $[\alpha]^{27}_D$ (c = 0.259, MeOH) -6.11; υ_{max}/cm^{-1}: 3344 (N-H), 2978 (saturated C-H), 1680 (amide C=O), 1519 (aromatic) and 1239 (C-O); δ_H (500 MHz, $CDCl_3$) 8.75 (s, 1H, indole

N*H*), 7.65 (d, 1H, *J* 7.5, indole C*H*-C-C), 7.38-7.28 (m, 6H, phenyl and indole C*H*-C-NH), 7.16 (t, 1H, *J* 7.5, indole C*H*-CH-C-NH), 7.10 (t, 1H, *J* 7.5, indole C*H*-CH-C-C), 6.96 (s, 1H, indole C*H*-NH), 5.73 (br.s, 1H, N*H*Cbz or N*H*CO-CH), 5.31 (br.s, 1H, N*H*-COO), 5.12 (s, 2H, CH_2-phenyl), 4.96 (br.t, 1H, *J* 5.5, N*H*Cbz or N*H*CO-CH), 4.40 (br.s, 1H, C*H*-CH_2- indole), 3.29 (dd, 1H, *J* 14, 4, CH_2-indole), 3.20-2.86 (m, 5H, CH_2-indole, CH_2-NH-CO and CH_2-NHCbz), 1.43 (s, 9H, C(CH_3)$_3$) and 1.22-1.08 (m, 4H, CH_2-CH_2-NHCbz and CH_2-CH_2-NHCO); δ_C (125 MHz, $CDCl_3$) 171.7 (*C*=O amide), 156.7, 155.5 (*C*=O carbamate), 136.6 (indole *C*-NH), 136.3 (phenyl *C*), 128.6, 128.2, 128.1 (phenyl *C*H), 127.3 (indole *C*-C-NH), 123.5 (indole *C*H-NH), 122.1 (indole *C*H-CH-C-C), 119.6 (indole *C*H-C-C), 118.8 (indole *C*H-CH-C-NH), 111.4 (indole *C*H-C-NH), 110.4 (indole *C*-CH_2), 80.0 (*C*(CH_3)$_3$), 66.8 (*C*H_2-phenyl), 55.4 (*C*H-CH_2-indole), 40.7, 39.0 (*C*H_2-NHCbz and *C*H_2-NH-CO), 28.9, 27.3, 26.5 (*C*H_2-indole, *C*H_2-CH_2-NHCbz and *C*H_2-CH_2-NHCO) and 28.4 (C(*C*H_3)$_3$); ESI *m/*z 100 %, 531.3 (MNa^+).

2.24, (*R*)-*O*-Benzyl *N*-(4-(2-*tert*butylcarbonylamino)-3-(1*H*-indol-3-yl)propanamido)pentylcarbamate

Acid **2.16** (0.56 g, 1.8 mmol) was dissolved in dichloromethane (20 mL) and cooled to 0°C. HATU (0.69 g, 1.8 mmol) was added and the mixture was stirred for 10 minutes. *N*-Z,1,5-Diaminopentane.HCl (0.50 g, 1.8 mmol) and triethylamine (0.80 mL, 5.7 mmol) were added and the reaction was stirred over night. The dichloromethane was removed *in vacuo*, the product dissolved in ethyl acetate and washed with a pH 2 buffer (3 × 15 mL) and ½ saturated $NaHCO_3$ (3 × 15 mL). The organic layer was dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate, 70:30 to 50:50) to give compound **2.24** as a white solid (0.56 g, 59 %); *mp* 114-115º C; $[\alpha]^{28}_D$ (c = 0.26,

MeOH) -7.88; υ_{max}/cm^{-1}: 3346 (N-H), 2936 (saturated C-H), 1678 (amide C=O), 1519 (aromatic) and 1246 (C-O); *Anal.* Calcd for $C_{29}H_{38}N_4O_5$: C, 66.64; H, 7.33; N, 10.72. Found: C, 66.35; H, 7.34; N, 10.62; δ_H (500 MHz, $CDCl_3$) 9.11 (br.s, 1H, indole N*H*), 7.67 (d, 1H, *J* 8, indole C*H*-C-C), 7.39-7.29 (m, 6H, phenyl & indole C*H*-C-NH), 7.17 (t, 1H, *J* 7.5, indole C*H*-CH-C), 7.11 (t, 1H, *J* 7.5, indole C*H*-CH-C-C), 6.98 (d, 1H, *J* 2, indole C*H*-NH), 5.62 (br.s, 1H, N*H*Cbz or N*H*-CH_2), 5.31 (br.s, 1H, N*H*-CH), 5.13 (s, 2H, C*H*$_2$-phenyl), 5.00 (br.t, 1H, *J* 5, N*H*Cbz or N*H*-CH_2), 4.42 (br.s, 1H, C*H*-CH_2- indole), 3.31 (dd, 1H, *J* 14, 4, C*H*$_2$-indole), 3.17-3.02 (m, 4H, C*H*$_2$-indole, C*H*$_2$-NHCO and C*H*$_2$-NHCbz), 2.99-2.88 (m, 1H, C*H*$_2$-NHCO or C*H*$_2$-NHCbz), 1.44 (s, 9H, C(C*H*$_3$)$_3$) 3.35 (quin., 2H, *J* 7, C*H*$_2$-CH_2-NH or C*H*$_2$-CH_2-NHCbz), 1.22-1.11 (m, 2H, C*H*$_2$-CH_2-NH or C*H*$_2$-CH_2-NHCbz) and 1.00-0.83 (m, 2H, C*H*$_2$-CH_2CH_2NHCbz); δ_C (125 MHz, $CDCl_3$) 171.7 (*C*=O amide), 156.8, 155.5 (*C*=O carbamate), 136.6 (indole *C*-NH), 136.4 (phenyl *C*), 128.6, 128.2, 128.16 (phenyl *C*H), 127.3 (indole *C*-C-NH), 123.5 (indole *C*H-NH), 122.0 (indole *C*H-CH-C-C), 119.6 (indole *C*H-C-C), 118.8 (indole *C*H-CH-C), 111.5 (indole *C*H-C-NH), 110.3 (indole *C*-CH_2), 80.0 (*C*(CH_3)$_3$), 66.8 (*C*H_2-phenyl), 55.3 (*C*H-CH_2-indole), 40.9, 39.0 (C*H*$_2$-NHCO and C*H*$_2$-NHCbz), 29.7, 29.0, 28.8, 23.5 (*C*H_2-indole, C*H*$_2$-CH_2-NH, C*H*$_2$-CH_2-NHCbz and C*H*$_2$-CH_2CH_2NHCbz) and 28.4 (C(*C*H$_3$)$_3$); ESI *m/*z 100 %, 545.3 (MNa^+).

2.25, (*R*)-*O*-Benzyl *N*-(4-(2-*tert*butylcarbonylamino)-3-(1*H*-indol-3-yl)propanamido)hexylcarbamate

Acid **2.16** (0.53 g, 1.7 mmol) was dissolved in dichloromethane (15 mL) and cooled to 0°C. HATU (0.66 g, 1.7 mmol) was added and the mixture was stirred for 10 minutes. *N*-Z,1,6-Diaminohexane.HCl (0.50 g, 1.7 mmol) and triethylamine (0.7 mL, 5 mmol) were added and the reaction was stirred over night. The dichloromethane

was removed *in vacuo*, the product was dissolved in ethyl acetate and washed with a pH 2 buffer (3 × 15 mL) and ½ saturated $NaHCO_3$ (3 × 15 mL). The organic layer was dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate, 70:30 to 40:60) to give compound **2.25** as a white solid (0.09 g, 10 %); *mp* 54-57 ° C; $[\alpha]^{27}_D$ (c = 0.502, MeOH) -2.59; υ_{max}/cm^{-1}: 3308 (N-H), 2932 (saturated C-H), 1694 (amide C=O), 1657 (aromatic) and 1247 (C-O); δ_H (500 MHz, $CDCl_3$) 8.92 (br.s, 1H, N*H*), 7.66 (d, 1H, *J* 7.5, indole C*H*-C-C), 7.38-7.29 (m, 6H, indole C*H*-C-NH and phenyl), 7.17 (t, 1H, *J* 7, indole C*H*-CH-C), 7.10 (t, 1H, *J* 7.5, indole C*H*-CH-C-C), 7.01 (s, 1H, indole C*H*-NH), 5.64 (br.s, 1H, N*H*-Cbz or N*H*-CO), 5.30 (br.s, 1H, N*H*-CH), 5.13 (s, 2H, CH_2-phenyl), 4.97 (br.s, 1H, N*H*-Cbz or N*H*-CO), 4.42 (br.s, 1H, C*H*-CH_2-indole), 3.31 (br.d, 1H, *J* 14, CH_2-indole), 3.17-3.05 (m, 3H, CH_2-indole, CH_2-NH-CO and CH_2-NHCbz), 3.03-2.95 (m, 1H, CH_2-NH-CO or CH_2-NHCbz), 1.89 (br.d, 1H, *J* 12, CH_2-NH-CO or CH_2-NHCbz), 1.44 (s, 9H, C(CH_3)$_3$) 1.48-1.36 (m, 3H, CH_2-CH_2NHCO, CH_2-CH_2CH_2NHCbz or CH_2-CH_2NHCbz), 1.22-1.12 (m, 3H, CH_2-CH_2NHCO, CH_2-CH_2CH_2NHCbz or CH_2-CH_2NHCbz) and 1.04-0.93 (m, 2H, CH_2-CH_2CH_2NHCO); δ_C (125 MHz, $CDCl_3$) 171.6 (*C*=O amide), 156.7, 155.5 (*C*=O carbamate), 136.6 (indole *C*-NH), 136.4 (phenyl *C*), 128.6, 128.2, 128.1 (phenyl), 127.4 (indole *C*-C-NH), 123.3 (indole *C*H-NH), 122.1 (indole *C*H-CH-C-C), 119.6 (indole *C*H-C-C), 118.8 (indole *C*H-CH-C), 111.4 (indole *C*H-C-NH), 110.4 (indole *C*-CH_2), 80.0 (*C*$(CH_3)_3$), 66.8 (*C*H_2-phenyl), 55.4 (*C*H-CH_2-indole), 40.9, 39.1 (*C*H_2-NH-CO or *C*H_2-NHCbz), 29.7, 28.9, 28.8, 26.0, (*C*H_2-indole, CH_2-CH_2NHCO, CH_2-CH_2CH_2NHCbz and CH_2-CH_2NHCbz), 25.8 (*C*H_2-CH_2CH_2NHCO) and 28.4 (C(*C*H_3)$_3$); ESI *m/*z 100 %, 559.3 (MNa^+).

2.26, (*S*)-*tert*-Butyl-6-(benzyloxycarbonylamino)-2-((*R*)-2-(*tert*-butoxycarbonylamino)-3-(1*H*-indol-3-yl)propanamido)hexanoate

Acid **2.16** (0.30 g, 0.99 mmol) was dissolved in dichloromethane (10 mL) and cooled to 0°C. HATU (0.38 g, 0.99 mmol) was added and the mixture was stirred for 10 minutes. H-Lysine(Z)-O^{t}Bu.HCl (0.37 g, 1.0 mmol) and triethylamine (0.4 mL, 3 mmol) were added and the reaction was stirred over night. The dichloromethane was removed *in vacuo*, the product was dissolved in ethyl acetate and washed with a pH 2 buffer (3 × 25 mL) and ½ saturated $NaHCO_3$ (3 × 25 mL). The organic layer was dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate, 90:10 to 50:50) to give compound **2.26** as a white solid (0.33 g, 52 %). The reaction was repeated on a scale of 5 mmol (2.61 g, 91 %); *mp* 131-132 °C; $[\alpha]^{27}_D$ (c = 0.0503, MeOH) -12.97; *Anal*. Calcd for $C_{34}H_{46}N_4O_7$: C, 65.57; H, 7.45; N, 9.00. Found: C, 65.51; H, 7.47; N, 8.93; υ_{max}/cm^{-1}: 3310 (N-H), 2948 (saturated C-H), 1700 (C=O) and 1661 (amide C=O); δ_H (400 MHz, *d*$_6$-DMSO) 10.79 (s, 1H, indole N*H*), 8.11 (d, 1H, *J* 7.5, N*H*-CH-COO^{t}Bu), 7.59 (d, 1H, *J* 8, indole C*H*-C-C), 7.37-7.31 (m, 6H, phenyl & indole C*H*-C-NH), 7.21 (t, 1H, *J* 5, N*H*-Cbz), 7.13 (s, 1H, indole C*H*-NH), 7.05 (t, 1H, *J* 7, indole C*H*-CH-C), 6.96 (t, 1H, *J* 7.5, indole C*H*-CH-C-C), 6.71 (d, 1H, *J* 8, N*H*-CH-CH_2), 4.98 (s, 2H, C*H*$_2$-phenyl), 4.26 (td, 1H, *J* 8, 5, C*H*-CH_2- indole), 4.02 (q, 1H, *J* 8, C*H*-COO^{t}Bu), 3.04 (dd, 1H, *J* 9, 4, C*H*$_2$-indole), 2.98-2.91 (m, 2H, C*H*$_2$-NHCbz), 2.89 (dd, 1H, *J* 8.5, 4, C*H*$_2$-indole), 1.57-1.64 (m,1H, C*H*$_2$ in $(CH_2)_4$ chain), 1.56-1.50 (m, 1H, C*H*$_2$ in $(CH_2)_4$ chain), 1.49-1.24 (m, 4H, CH_2 in $(CH_2)_4$ chain), 1.39 (s, 9H, CHCOOC(C*H*$_3$)$_3$) and 1.31 (s, 9H, NHCOOC(C*H*$_3$)$_3$); δ_C (100 MHz, *d*$_6$-DMSO) 172.0, 171.2 (*C*=O ester and amide) 156.0, 155.0 (*C*=O carbamate), 137.2 (indole *C*-

NH), 136.0 (phenyl *C*), 128.3, 127.7 (phenyl *C*H), 127.4 (indole *C*-C-NH), 123.6 (indole *C*H-NH), 120.8 (indole *C*H-CH-C-C), 118.5 (indole *C*H-C-C), 118.0 (indole *C*H-CH-C), 111.2 (indole *C*H-C-NH), 110.1 (indole *C*-CH_2), 80.5, (CHCOO*C*$(CH_3)_3$), 77.9 (NHCOO*C*$(CH_3)_3$), 65.1 (*C*H_2-phenyl), 54.9 (*C*H-NHCOO), 52.6 (*C*H-COO^{t}Bu), 40.1 (*C*H_2–indole), 38.9 (*C*H_2-NHCbz), 30.8, 28.9, 22.3 (*C*H_2 from $CH_2)_4$ chain), 28.1 (NHCOOC(*C*$H_3)_3$) and 27.4 (CHCOOC(*C*$H_3)_3$); HR ESI *m/*z ($C_{34}H_{46}N_4O_7H^+$ requires 623.3439) found 623.3457.

2.27, (*R*)-2-Amino-3-(1*H*-indol-3-yl)-*N*-propylpropanamide

Compound **2.22** (3.04 g, 8.80 mmol) was dissolved in MeOH (50 mL), methane sulfonic acid (1.70 mL, 26.4 mmol) was added and the reaction was stirred for 20 hours and determined to be completed by TLC. The reaction was quenched with triethylamine (6.1 mL, 44 mmol) and reduced *in vacuo* to give compound **2.27** as a colourless oil which was not isolated. This compound has previously been reported but with no characterisation data.[235]

2.28, (*R*)-*O*-Benzyl *N*-(4-(2-amino)-3-(1*H*-indol-3-yl)propanamido) butylcarbamate

Carbamate **2.23** (0.31 g, 0.61 mmol) was dissolved in MeOH (15 mL), methane sulfonic acid (145 μL, 1.83 mmol) was added and the reaction was stirred for 48 hours and determined to be completed by TLC. The reaction was quenched with triethylamine (0.5 mL, 3 mmol) and reduced *in vacuo* to give compound compound **2.28** as a colourless oil which was not isolated.

2.29, (*R*)-*O*-Benzyl *N*-(5-(2-amino)-3-(1*H*-indol-3-yl)propanamido) pentylcarbamate

Carbamate **2.24** (0.3 g, 0.5 mmol) was dissolved in MeOH (3 mL), methane sulfonic acid (97 μL, 1.5 mmol) was added and the reaction was stirred for 24 hours and determined to be completed by TLC. The reaction was quenched with triethylamine (0.35 mL, 2.5 mmol) and reduced *in vacuo* to give compound **2.29** as a colourless oil which was not isolated.

2.30, (*R*)-*O*-Benzyl *N*-(6-(2-amino)-3-(1*H*-indol-3-yl)propanamido) hexylcarbamate

Carbamate **2.25** (0.09 g, 0.2 mmol) was dissolved in MeOH (3 mL), methane sulfonic acid (42 μL, 0.75 mmol) was added and the reaction was stirred for 24 hours and determined to be completed by TLC. The reaction was quenched with triethylamine (0.18 mL, 1.3 mmol) and reduced *in vacuo* to give compound **2.30** as a colourless oil which was not isolated.

2.31, (*S*)-*tert*-Butyl 2-((*R*)-2-amino-3-(1*H*-indol-3-yl)propanamido)-6-(benzyloxycarbonylamino)hexanoate

Carbamate **2.26** (4.35 g, 6.99 mmol) was dissolved in MeOH (150 mL), methane sulfonic acid (1.36 mL, 21.0 mmol) was added, the reaction was stirred for 72 hours at room temperature. The reaction was quenched with triethylamine (4.9 mL, 35 mmol) and reduced *in vacuo* to give compound **2.31** as a brown oil, by ^{1}H NMR yield was determined as 100 % as product was pure; δ_H (400 MHz, d_6-DMSO) 10.99 (s, 1H, N*H* indole), 8.55 (1H, d, *J* 7.5, N*H*-CHCOO), 7.63 (d, 1H, *J* 8, indole C*H*-C-C), 7.40-7.27 (m, 6H, indole C*H*-C-NH & phenyl), 7.23 (s, 1H, indole C*H*-NH), 7.08 (t, 1H, *J* 7.5, indole C*H*-CH-C), 6.99 (t, 1H, *J* 7.5, indole C*H*-CH-C-C), 4.99 (s, 2H, CH_2-phenyl), 4.08 (q, 1H, *J* 8, C*H*-COO), 3.91 (t, 1H, *J* 6.5, C*H*-NH_2), 3.19 (dd, 1H, *J* 14.5, 6.5, CH_2-indole), 2.98 (dd, 1H, *J* 14.5, 6.5, CH_2-indole), 1.41 (s, 9H,

$C(CH_3)_3$), 1.64-1.22 (m, 6H, C<u>*H*</u>$_2$ of $(CH_2)_4$ chain) and 1.05-0.98 (m, 2H, C<u>*H*</u>$_2$ of $(CH_2)_4$ chain); δ_C (100 MHz, d_6-DMSO) 170.9 and 170.4 (ester and amide <u>*C*</u>=O), 156.1 (carbamate <u>*C*</u>=O), 137.2 (indole <u>*C*</u>-NH), 136.2 (phenyl <u>*C*</u>), 128.3, 127.6 (phenyl <u>*C*</u>H), 127.5 (indole <u>*C*</u>-C-NH), 124.6 (indole <u>*C*</u>H-NH), 120.9 (indole <u>*C*</u>H-C-C), 118.4, 118.3 (indole <u>*C*</u>H-CH-C and <u>*C*</u>H-CH-C-C), 111.4 (indole <u>*C*</u>H-CNH), 107.9 (indole <u>*C*</u>-CH_2), 80.8 (<u>*C*</u>$(CH_3)_3$), 65.1 (<u>*C*</u>H_2-phenyl), 52.5 (<u>*C*</u>H-NH_2), 52.0 (<u>*C*</u>H-COO), 45.7 (<u>*C*</u>H_2-indole), 39.7 (C(<u>*C*</u>$H_3)_3$), 28.9, 28.7 (<u>*C*</u>H_2-CH and <u>*C*</u>H_2-NHCbz) and 22.6, 22.25 (<u>*C*</u>H_2-CH_2CH and CH_2-CH_2NHCbz); HR ESI *m/z* ($C_{29}H_{38}N_4O_5H^+$ requires 523.2915) found 523.2916.

2.32, (*R*)-3-(1*H*-Indol-3-yl)-2-(4-methylphenylsulfonamido)-*N*-propyl propanamide

Amine **2.27** (7 mmol) was dissolved in dichloromethane, *para*-toluenesulfonyl chloride (1.42 g, 7.45 mmol) and triethylamine (2.9 mL, 21 mmol,) were added, the reaction was stirred for 15 hours. The dichloromethane was removed *in vacuo*, the product dissolved in ethyl acetate and washed with a pH 2 buffer (3 × 25 mL). The organic layer was dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate:MeOH, 62.5:37.5:0 to 0:80:20) to give compound **2.32** as a white solid (0.59 g, 21 %); *mp* 69-71º C; $[\alpha]^{20}_D$ (c = 0.244, MeOH) 50.11; υ_{max}/cm^{-1}: 3389 (N-H), 2965 (saturated C-H), 1648 (amide C=O) and 1322, 1156 (SO_2); δ_H (500 MHz, $CDCl_3$) 8.40 (s, 1H, N<u>*H*</u> indole), 7.43 (d, 2H, *J* 7, C<u>*H*</u>-CSO_2), 7.28 (t, 2H, *J* 8, indole C<u>*H*</u>-C-NH and C<u>*H*</u>-C-C), 7.15 (t, 1H, *J* 7.5, indole C<u>*H*</u>-CH-C), 7.04-7.95 (m, 3H, C<u>*H*</u>-C-CH_3 and indole C<u>*H*</u>-CH-C-C), 6.91 (s, 1H, indole C<u>*H*</u>-NH), 6.40 (t, 1H, *J* 5, N<u>*H*</u>-CH_2CH_2), 5.19 (d, 1H, *J* 6, N<u>*H*</u>-CH), 3.89 (q, 1H, *J* 6, C<u>*H*</u>-CH_2-indole), 3.18-3.01 (m, 4H, CH_2-indole and C<u>*H*</u>$_2$-CH_2CH_3), 2.31 (s, 3H, C<u>*H*</u>$_3$-aryl), 1.36 (sextet, 1H, *J* 7.5, C<u>*H*</u>$_2$-CH_3) and 0.79 (t, 1H,

J 7.5, C*H*$_3$-CH$_2$); δ_C (125 MHz, $CDCl_3$) 170.8 (*C*=O), 143.8 (*C*-CH_3), 136.4 (indole *C*-NH), 135.2 (*C*-SO_2), 129.6 (*C*-CCH_3), 126.9 (*C*-CSO_2), 126.8 (indole *C*-C-NH), 123.4 (indole *C*H-NH), 122.4 (indole *C*H-CH-C-C), 119.8 (indole *C*H-C-C), 118.5 (indole *C*H-CH-C), 111.3 (indole *C*H-C-NH), 109.3 (indole *C*-CH_2), 57.0 (*C*H-CH_2-indole), 41.4 (*C*H_2-NH), 28.6 (*C*H_2-indole), 22.5 (*C*H_2-CH_3), 21.6 (*C*H_3-aryl) and 11.23 (*C*H_3-CH_2); ESI *m/*z 100 %, 422.2 (MNa^+) and 50 %, 821.2 (M_2Na^+).

2.33, (*R*)-*N*-(3-(1*H*-Indol-3-yl)-1-oxo-1-(propylamino)propan-2-yl)biphenyl-4-carboxamide

4-Biphenylcarboxylic acid (1.29 g, 6.51 mmol) was dissolved in dichloromethane and cooled to 0 °C. HATU (2.35 g, 6.18 mmol) was added and the reaction stirred for 10 minutes. Amine **2.27** (5.7 mmol) was dissolved dichloromethane and triethylamine (2.4 mL, 17 mmol) were added to the reaction which was stirred for 48 hours. The dichloromethane was removed *in vacuo*, and the product was dissolved in ethyl acetate and washed with a pH 2 buffer (3 × 15 mL), 0.1 M HCl (3 × 15 mL) and sodium bicarbonate (3 × 15 mL). The organic layer was dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate:MeOH, 60:40:0 to 0:80:20) to give compound **2.33** as a brown solid (1.00 g, 63 %); *mp* 188-189 ° C; υ_{max}/cm^{-1}: 3435, 3316 (N-H), 2932 (saturated C-H), 1625 (C=O amide) and 1529 (aromatic); δ_H (500 MHz, $CDCl_3$) 8.26 (s, 1H, indole N*H*), 7.82 (d, 1H, *J* 8, indole C*H*-C-C), 7.81 (d, 2H, *J* 8, biphenyl C*H*-C-CO), 7.62 (d, 2H, *J* 9, biphenyl C*H*-CH-C-CO and C*H*-C-aryl), 7.60 (d, 2H, *J* 7.5, biphenyl C*H*-CH-C-CO and C*H*-C-aryl), 7.46 (t, 2H, *J* 7.5, biphenyl C*H*-CH-C-aryl), 7.40-7.36 (m, 2H, indole C*H*-C-NH and biphenyl C*H*-CH-CH-C-aryl), 7.23-7.14 (m, 3H, indole C*H*-CH-C, C*H*-CH-C-C and N*H*-CO-biphenyl), 7.11 (s, 1H, indole C*H*-NH), 5.83 (s, 1H, N*H*-CH_2), 4.94 (dt, 1H, *J* 13, 5, C*H*-CH_2-indole), 3.50

(dd, 1H, *J* 9, 5, C*H*$_2$-indole), 3.24 (dd, 1H, *J* 13, 9, C*H*$_2$-indole), 3.11 (quin., 1H, *J* 7, C*H*$_2$-Et), 3.05 (quin., 1H, *J* 7, C*H*$_2$-CH$_2$-CH$_3$), 1.33-1.24 (m, 2H, C*H*$_2$-CH$_3$) and 0.73 (t, 3H, *J* 7, C*H*$_3$); δ_C (125 MHz, $CDCl_3$) 171.2 (*C*=O), 166.9 (*C*=O), 144.6 (biphenyl *C*-phenyl), 140.0 (biphenyl *C*-aryl), 136.3 (indole *C*-NH), 132.4 (biphenyl *C*-CO), 128.9 (biphenyl *C*H-CHC-aryl), 128.1 (biphenyl *C*H-C), 127.7 (biphenyl *C*H-C-aryl), 127.4 (biphenyl *C*H-C-phenyl), 127.1 (biphenyl *C*H-CHCH-C-aryl), 127.2 (indole *C*-C-NH), 123.2 (indole *C*H-NH), 122.4 (indole *C*H-CH-C-C), 119.9 (indole *C*H-C-C), 119.1 (indole *C*H-CH-C), 111.3 (indole *C*H-C-NH), 111.0 (indole *C*-CH$_2$), 54.5 (*C*H-CH$_2$-indole), 41.3 (*C*H$_2$-NHCO), 28.7 (*C*H$_2$-indole), 22.5 (*C*H$_2$-CH$_3$) and 11.2 (*C*H$_3$); ESI *m/*z 100 %, 448.2 (MNa^+) and 30 %, 873.3 (M_2Na^+).

2.34, (*R*)-*O*-Benzyl *N*-(4-(3-(1*H*-Indol-3-yl)-2-(1-tosylpiperidine-4-carboxamido)propanamido)butylcarbamate)

Acid **2.11** (0.34 g, 1.2 mmol) was dissolved in dichloromethane (10 mL) and cooled to 0°C. HATU (0.46 g, 1.2 mmol) was added and the mixture was stirred for 10 minutes. Amine **2.28** (1.2 mmol) in dichloromethane and triethylamine (0.50 mL, 3.6 mmol) were added and the reaction was stirred over night. The dichloromethane was removed *in vacuo*, the product was dissolved in ethyl acetate and washed with a pH 2 buffer (3 × 10 mL) and ½ saturated $NaHCO_3$ (3 × 10 mL). The organic layer was dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate, 95:5 to 0:100) to give compound **2.34** as a colourless oil (0.36 g, 47 %); υ_{max}/cm^{-1}: 3302 (N-H), 2939 (saturated C-H), 1698, 1644 (C=O), 1644, 1328, 1160 (SO_2) and 1251 (C-O); δ_H (400 MHz, $CDCl_3$) 8.92 (br.s, 1H, indole N*H*), 7.58 (d, 3H, *J* 7, indole C*H*-C-C and C*H*-CSO_2), 7.38-7.24 (m, 8H, indole C*H*-C-NH, C*H*-CCH_3 and phenyl), 7.10 (t, 1H, *J* 7.5, indole C*H*-CH-C), 7.02 (t, 1H, *J* 7.5, indole C*H*-CH-C-C), 6.93 (s, 1H, indole C*H*-NH), 6.71 (d,

1H, *J* 6, N*H*-CH), 6.29 (br.t, 1H, *J* 5, N*H*Cbz or N*H*-CO), 5.20 (br.t, 1H, *J* 5, N*H*Cbz or N*H*-CO), 5.09 (s, 2H, C*H*$_2$-phenyl), 4.70 (br. q, 1H, *J* 7, C*H*-CH$_2$-indole), 3.66 (br.t, 2H, *J* 11, equatorial C*H*$_2$-NSO$_2$), 3.18 (dd, 2H, *J* 14, 5, C*H*$_2$-indole), 3.12-2.92 (m, 3H, C*H*$_2$-NHCbz & C*H*$_2$-NHCO), 2.89-2.77 (m, 1H, C*H*$_2$-NHCO), 2.41 (s, 3H, C*H*$_3$-aryl), 2.22-2.12 (m, 2H, *J* 11, axial C*H*$_2$-NSO$_2$), 1.98-1.88 (m, 1H, C*H*-CH$_2$CH$_2$N), 1.76-1.37 (m, 4H, C*H*$_2$-CH$_2$NSO$_2$) and 1.21-1.05 (m, 4H, C*H*$_2$-CH$_2$-NHCO and C*H*$_2$-CH$_2$-NHCbz); δ_C (100 MHz, $CDCl_3$) 173.8, 171.5 (*C*=O amide), 156.9 (*C*=O carbamate), 143.8 (*C*-CH$_3$), 136.6 (indole *C*-NH), 136.3 (phenyl *C*), 133.0 (*C*-SO$_2$), 129.8 (*C*H-CCH$_3$), 128.6, 128.2 (phenyl *C*H), 128.1 (*C*H-CSO$_2$), 127.7 (phenyl *C*H), 127.3 (indole *C*-C-NH), 123.4 (indole *C*H-NH), 122.1 (indole *C*H-CH-C-C), 119.5 (indole *C*H-C-C), 118.8 (indole *C*H-CH-C), 111.5 (indole *C*H-C-NH), 110.3 (indole *C*-CH$_2$), 66.7 (*C*H$_2$-phenyl), 53.9 (*C*H-CH$_2$-indole), 45.5 (*C*H$_2$-N), 41.8 (*C*H-CH$_2$CH$_2$N), 40.7 (*C*H$_2$-NHCbz), 39.1 (*C*H$_2$-NHCO), 29.0, 28.0, (*C*H$_2$-indole and *C*H$_2$-CH$_2$N), 27.5, 26.4 (*C*H$_2$-CH$_2$NHCbz, *C*H$_2$-CH$_2$NHCO) and 21.6 (CH$_3$); ESI *m/*z 100 %, 696.3 (MNa+) and 35 %, 674.5 (MH^+).

2.35, (*R*)-*N*-(3-(1*H*-Indol-3-yl)-1-oxo-1-(5-(2-phenoxyacetamido)pentylamino) propan-2-yl)-1-(*meta*-tolylsulfonyl)piperidine-4-carboxamide

Acid **2.10** (0.20 g, 0.71 mmol) was dissolved in dichloromethane (4 mL) and cooled to 0° C. HATU (0.19 g, 0.51 mmol) was added and the mixture was stirred for 1 hour. Amine **2.29** (0.5 mmol) in dichloromethane and triethylamine (0.20 mL, 1.4 mmol) were added and the reaction was stirred over night. The dichloromethane was removed *in vacuo*, the product was dissolved in ethyl acetate and washed with a pH 2 buffer (3 × 5 mL), 0.1 M HCl (3 × 5 mL) and ½ saturated $NaHCO_3$ (3 × 5 mL). The organic layer was dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate, 50:50 to

0:100) to give compound **2.35** as a white solid (0.17 g, 49 %) *mp* 69-70 °C; υ_{max}/cm^{-1}: 3289 (N-H), 2928 (saturated C-H), 1697, 1639 (C=O), 1526 (aromatic), 1329, 154 (SO_2) and 1248 (C-O); δ_H (400 MHz, $CDCl_3$) 9.21 (indole N*H*), 7.61 (d, 1H, *J* 7.5, indole C*H*-C-C), 7.54 (s, 1H, C*H*-CSO_2), 7.51 (t, 1H, *J* 5, C*H*-$CHCCH_3$), 7.40 (d, 2H, *J* 5, C*H*-CCH_3 and C*H*-$CHCHCCH_3$), 7.35-7.28 (m, 6H, indole C*H*-C-NH and phenyl), 7.13 (t, 1H, *J* 7.5, indole C*H*-CH-C), 7.03 (t, 1H, *J* 7.5, indole C*H*-CH-C-C), 6.94 (d, 1H, *J* 2, indole C*H*-NH), 6.72 (d, 1H, *J* 7.5, N*H*-CH), 6.11 (br.t, 1H, *J* 5.5, N*H*-COCH or N*H*Cbz), 5.24 (br.t, 1H, *J* 5.5, N*H*-COCH or N*H*Cbz), 5.10 (s, 2H, CH_2-phenyl), 4.72 (q, 1H, *J* 7, C*H*-CH_2-indole), 3.72-3.65 (m, 2H, equatorial CH_2-N), 3.20 (dd, 1H, *J* 15.4, 6, CH_2-indole), 3.15-3.00 (m, 4H, CH_2-NHCbz, CH_2-NHCO and CH_2-indole), 2.89-2.78 (m, 1H, CH_2-NHCO), 2.42 (s, 3H, CH_3), 2.25-2.17 (m, 2H, axial CH_2-N), 1.98-1.91 (m, 1H, C*H*-CCO), 1.70 (d, 4H, *J* 11, CH_2-CH_2N), 1.34 (br.t, 2H, *J* 7, CH_2-CH_2NHCbz), 1.16 (quin, 2H, *J* 7, CH_2-CH_2NHCO) and 1.00-0.84 (m, 2H, CH_2-$(CH_2)_2$NHCbz); δ_C (100 MHz, $CDCl_3$) 173.7, 171.4 (*C*=O), 156.9 (*C*=O carbamate), 139.3 (*C*-CH_3), 136.6 (*C*-SO_2), 136.4 (indole *C*-NH), 135.8 (phenyl *C*), 133.7 (*C*H-CCH_3), 129.0 (*C*H-CH-CCH_3), 128.6, 128.1 (phenyl *C*H), 127.9 (*C*H-CSO_2), 127.3 (indole *C*-C-NH), 124.8 (*C*H-CH-$CHCCH_3$), 123.4 (indole *C*H-NH), 122.0 (indole *C*H-CH-C-C), 119.4 (indole *C*H-C-C), 118.7 (indole *C*H-CH-C), 111.6 (indole *C*H-C-NH), 110.2 (indole *C*-CH_2), 66.7 (*C*H_2-phenyl), 53.9 (*C*H-CH_2-indole), 45.5 (*C*H_2-N), 41.8 (*C*H-CH_2-CH_2-N), 40.9 (*C*H_2-NH-Cbz), 39.2 (*C*H_2-NH-CO), 29.7, 29.1, 28.7, 28.1 (*C*H_2-indole, *C*H_2-CH_2N, *C*H_2-CH_2NHCbz & *C*H_2-CH_2NHCO), 23.6 (*C*H_2-$(CH_2)_2$-NH-Cbz) and 21.4 (*C*H_3); ESI *m/*z 100 %, 710.2 (MNa^+) and 39 %, 423.3 (MH^+-$C_{13}H_{16}NO_3S$); HR ESI *m/*z ($C_{37}H_{45}N_5O_6SNa^+$ requires 710.2983) found 710.2975.

2.36, (*R*)-*O*-Benzyl 5-(3-(1*H*-indol-3-yl)-2-(1-tosylpiperidine-4-carboxamido) propanamido)pentylcarbamate

Acid **2.11** (0.20 g, 0.71 mmol) was dissolved in dichloromethane (10 mL) and cooled to 0° C. HATU (0.26 g, 0.69 mmol) was added and the mixture was stirred for 10 minutes. Amine **2.29** (0.7 mmol) in dichloromethane and triethylamine (2.1 mmol, 0.29 mL) were added and the reaction was stirred over night. The dichloromethane was removed *in vacuo*, the product was dissolved in ethyl acetate was washed with a pH 2 buffer (3 × 10 mL) and ½ saturated $NaHCO_3$ (3 × 10 mL). The organic layer was dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate, 95:5 to 20:80) to give compound **2.36** as a white-yellow solid (0.12 g, 25 %); *mp* 115-117 º C; $[\alpha]^{30}_D$ (c = 0.25, MeOH) -0.1; υ_{max}/cm^{-1}: 3293 (N-H), 2927 (saturated C-H), 1698, 1633 (C=O), 1326, 1152 (SO_2) and 1246 (C-O); *Anal.* Calcd for $C_{37}H_{45}N_5O_6S$: C, 64.61; H, 6.59; N, 10.18. Found: C, 64.01; H, 6.59; N, 10.18; δ_H (400 MHz, $CDCl_3$) 9.27 (s, 1H, indole N*H*), 7.56 (d, 2H, *J* 8, indole C*H*-C-C and C*H*-CSO_2), 7.33-7.23 (m, 8H, indole C*H*-C-NH, C*H*-CCH_3 and phenyl), 7.11 (1H, t, *J* 7.5, indole C*H*-CH-C), 6.99 (t, 1H, *J* 7.5, indole C*H*-CH-C-C), 6.90 (s, 1H, indole C*H*-NH), 6.79 (d, 1H, *J* 7.5, N*H*-CH), 6.27 (br.t, 1H, *J* 4.5, N*H*Cbz or N*H*-CO), 5.34 (br.t, 1H, *J* 5, N*H*Cbz or N*H*-CO), 5.07 (s, 2H, CH_2-phenyl), 4.71 (q, 1H, *J* 7, C*H*-CH_2-indole), 3.62 (br.t, 2H, *J* 10, equatorial CH_2-N), 3.22-2.94 (m, 5H, CH_2-indole, CH_2-NHCbz and CH_2-NHCO), 2.85-2.73 (m, 1H, CH_2-NHCbz or CH_2-NHCO), 2.38 (s, 3H, CH_3), 2.17-2.06 (m, 2H, axial CH_2-N), 1.94-1.84 (m, 1H, C*H*-CH_2CH_2N), 1.64 (m, 4H, CH_2-CH_2-N), 1.35-1.26 (br.t, 2H, CH_2-CH_2-NHCO), 1.13 (td, 2H, *J* 7.5, 1.5, CH_2-CH_2CH_2-NHCO or CH_2-CH_2CH_2NHCbz) and 0.96-0.79 (m, 2H, CH_2-CH_2CH_2-NHCO or CH_2-CH_2CH_2NHCbz); δ_C (100 MHz, $CDCl_3$) 173.8, 171.5 (*C*=O amide),

156.9 (carbamate <u>C</u>=O), 143.8 (<u>C</u>-CH_3), 136.6 (indole <u>C</u>-NH), 136.4 (phenyl <u>C</u>), 132.8 (<u>C</u>-SO_2), 129.8 (<u>C</u>H-CCH_3), 128.6, 128.1 (phenyl <u>C</u>H), 128.0 (<u>C</u>H-CSO_2), 127.7 (phenyl <u>C</u>H), 127.3 (indole <u>C</u>-C-NH), 123.4 (indole <u>C</u>H-NH), 121.9 (indole <u>C</u>H-CH-C-C), 119.3 (indole <u>C</u>H-C-C), 118.7 (C3), 111.6 (indole <u>C</u>H-C-NH), 110.1 (indole <u>C</u>-CH_2), 66.6 (<u>C</u>H_2-phenyl), 53.9 (<u>C</u>H-CH_2-indole), 45.5 (<u>C</u>H_2-NSO_2), 41.7 (<u>C</u>H-CH_2CH_2N), 40.9, 39.2 (<u>C</u>H_2-NHCbz and <u>C</u>H_2-NHCO), 29.7, 29.1, 28.7, 28.6 (<u>C</u>H_2-indole, <u>C</u>H_2-CH_2N, <u>C</u>H_2-CH_2NHCbz and <u>C</u>H_2-CH_2NHCO), 23.7 (<u>C</u>H_2-CH_2CH_2NHCbz) and 21.6 (CH_3); ESI *m/*z 100 %, 710.4 (MNa^+) and 62 %, 688.3 (MH^+).

2.37, (*S*)-*tert*-Butyl 2-((*R*)-3-(1*H*-indol-3-yl)-2-(1-(*meta*-tolylsulfonyl)piperidine-4-carboxamido)propanamido)-6-(benzyloxycarbonylamino)hexanoate

Acid **2.10** (1.13 g, 3.99 mmol) was dissolved in dichloromethane (30 mL) and cooled to 0 ºC. HATU (1.70 g, 4.47 mmol) was added and the reaction was stirred for 4 hours. Amine **2.31** (4 mmol) in dichloromethane (20 mL) and with triethylamine (1.7 mL, 12 mmol) were added and the reaction was stirred for 48 hours. The dichloromethane was removed *in vacuo*, and the product was dissolved in ethyl acetate was washed with a pH 2 buffer (3 × 20 mL), 0.1 M HCl (3 × 20 mL), and ½ saturated $NaHCO_3$ (3 × 20 mL). The product was purified by silica column chromatography (petroleum ether:ethyl acetate:MeOH 80:20:0 to 0:70:30) to give compound **2.37** as a white solid (1.61g, 51 %); *mp* 76-78 ºC; $[\alpha]^{24}_D$ (c = 0.505, MeOH) - 11.19; υ_{max}/cm^{-1}: 3298 (N-H), 2932 (saturated C-H), 1705 (C=O ester), 1644 (C=O amide), 1520 (aromatic), 1332, 1155 (SO_2) and 1249 (C-O); δ_H (400 MHz, $CDCl_3$) 9.28 (br.s, 1H, N<u>H</u> indole), 7.67 (d, 1H, *J* 8, indole C<u>H</u>-C-C), 7.54 (s, 1H, C<u>H</u>-CSO_2), 7.52 (d, 1H, *J* 6.5, C<u>H</u>-CHCH-CCH_3),7.4-7.37 (m, 2H, C<u>H</u>-CCH_3 and C<u>H</u>-$CHCCH_3$), 7.35-7.30 (m, 5H, phenyl), 7.14 (t, 1H, *J* 8, indole C<u>H</u>-CH-C),

7.07 (t, 1H, *J* 7.5, indole C*H*-CH-C-C), 6.94 (d, 1H, *J* 1.5, indole C*H*-NH), 6.39 (d, 1H, *J* 7, N*H*-CH), 6.0 (d, 1H, *J* 7.5, N*H*-CH), 5.12 (dd, 1H, *J* 14, 12, CH_2-phenyl), 5.05 (t, 1H, *J* 6, N*H*-Cbz), 4.74 (br.q, 1H, *J* 7, C*H*-CH_2-indole), 4.26 (q, 1H, *J* 6.5, C*H*-COO^{t}Bu), 3.73 (d, 1H, *J* 14, CH_2-N equatorial), 3.69 (d, 1H, *J* 14, CH_2-N equatorial), 3.24 (dd, 1H, *J* 14, 4.5, CH_2-indole), 3.15-2.93 (m, 3H, CH_2-indole & CH_2-NH-Cbz), 2.42 (s, 3H, CH_3), 2.29 (td, 2H, *J* 11.5, 3, CH_2-N axial), 2.00-1.95 (m, 2H, CH_2-CH-COO^{t}Bu), 1.85-1.69 (m, 4H, CH_2-CH_2-N), 1.51-1.39 (m, 1H, CH_2-CH_2-NH-Cbz), 1.35 (s, 9H, C(CH_3)$_3$), 1.36-1.28 (m, 1H, CH_2-CH_2-NH-Cbz), 0.86-0.75 (m, 1H, CH_2-CH_2-CH-COO^{t}Bu) and 0.72-0.65 75 (m, 1H, CH_2-CH_2-CH-COO^{t}Bu); δ_C (100 MHz, $CDCl_3$) 173.6, 170.9, 170.7 (*C*=O ester and amide), 156.9 (*C*=O carbamate), 139.3 (*C*-CH_3), 136.5 (*C*-SO_2), 136.0 (indole *C*-NH), 133.7 (*C*-CH_3), 129.0 (*C*H-CCH_3), 128.2, 128.1 (phenyl *C*H), 127.9 (*C*H-CSO_2), 127.1 (indole *C*-C-NH), 124.8 (*C*H-CHCHCCH_3), 123.2 (indole *C*H-NH), 122.1 (indole *C*H-C-C), 119.6 (indole *C*H-CH-C), 118.7 (indole *C*H-CH-C-C), 111.6 (indole *C*H-C-NH), 110.0 (indole *C*-CH_2), 82.8 (*C*(CH_3)$_3$), 53.9 (*C*H-CH_2-indole), 52.6 (*C*H-COO^{t}Bu), 45.5 (*C*H_2-N), 41.9 (*C*H-CH_2CH_2N), 40.8 (*C*H_2-NHCbz), 31.7, 29.8, 29.2, 27.98, 27.92 (*C*H_2-indole, *C*H_2-CH_2-N, *C*H_2-CH_2NHCbz, *C*H_2-CHCOO^{t}Bu) and 21.6 (*C*H_2-CH_2CHCOO^{t}Bu); ESI *m/*z 100 %, 810.3 (MNa^+).

2.38, (*S*)-*tert*-Butyl 2-((*R*)-3-(1*H*-indol-3-yl)-2-(1-tosylpiperidine-4-carboxamido) propanamido)-6-(benzyloxycarbonylamino)hexanoate

Acid **2.11** (0.10 g, 3.5 mmol) was dissolved in dichloromethane (30 mL) and cooled to 0 ºC. HATU (1.39 g, 3.66 mmol) was added and the reaction was stirred for 10 minutes. Amine **2.31** (3.5 mmol) in dichloromethane (20 mL) and triethylamine (1.5 mL, 10.7 mmol) were added and the reaction was stirred for 48 hours. The dichloromethane was removed *in vacuo*, the product dissolved in ethyl acetate was

washed with a pH 2 buffer (3 × 50 mL) and ½ saturated $NaHCO_3$ (3 × 50 mL). The organic layer was dried over Na_2CO_3 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate:MeOH 80:20:0 to 0:90:10) to give compound **2.38** (1.75g, 63 %); *mp* 155-157 ºC; $[\alpha]^{25}_{D}$ (c = 1, chloroform) -3.70; υ_{max}/cm^{-1}: 3333 (N-H), 2957 (saturated C-H), 1704 (ester/carbamate C=O), 1663 (amide C=O), 1527 (aromatic) and 1338, 1151 (SO_2) and 1250 (ester C-O); δ_H (400 MHz, $CDCl_3$) 9.36 (s, 1H, indole N*H*), 7.65 (d, 1H, *J* 7.5, indole C*H*-C-C), 7.60 (d, 2H, *J* 8, C*H*-CSO_2), 7.36-7.28 (m, 8H, phenyl, indole C*H*-C-NH and C*H*-C-CH_3), 7.13 (t, 1H, *J* 7.5, indole C*H*-CH-C), 7.06 (t, 1H, *J* 7, indole C*H*-CH-C-C), 6.95 (s, 1H, indole C*H*-NH), 6.40 (d, 1H, *J* 7.5, N*H*-$CHCH_2$-indole), 6.01 (d, 1H, *J* 7.5, N*H*-CH-COO), 5.14 (d, 1H, *J* 12, C*H*$_2$-phenyl), 5.11 (d, 1H, *J* 12, C*H*$_2$-phenyl), 5.11 (t, 1H, *J* 5, N*H*Cbz), 4.75 (br.q, 1H, *J* 5, C*H*-CH_2-indole), 4.31 (br.q, 1H, *J* C*H*-COO), 3.70 (br.t, 2H, *J* 11 equatorial C*H*-NSO_2), 3.22 (dd, 1H, *J* 14, 4.5, C*H*$_2$-indole), 3.17-2.91 (m, 3H, C*H*$_2$-NHCbz & C*H*$_2$-indole), 2.42 (s, 3H, C*H*$_3$-aryl), 2.31-2.18 (m, 2H, axial C*H*$_2$-NSO_2), 2.00-1.94 (m, 1H, piperidine C*H*-CONH), 1.85-1.80 (m, 2H, C*H*$_2$-CH_2-NSO_2), 1.79-1.67 (m, 2H, C*H*$_2$-CH_2-NSO_2 and C*H*$_2$-CH), 1.47-1.40 (m, 1H, C*H*$_2$-CH), 1.36-1.22 (m, 2H, C*H*$_2$-CH_2-NHCbz), 1.35 (s, 9H, (C*H*$_3$)$_3$), 0.84-0.76 (m, 1H, C*H*$_2$-CH_2-CH) and 0.72-0.64 (m, 1H, C*H*$_2$-CH_2-CH); δ_C (100 MHz, $CDCl_3$) 173.0 (*C*=O ester), 170.6, 170.2 (*C*=O amide), 156.3 (*C*=O carbamate), 143.6 (*C*-CH_3), 136.5 (indole *C*-NH), 133.1(*C*-SO_2), 129.7 (*C*H-C-CH_3), 128.6, 128.2 (phenyl *C*H), 128.1 (*C*H-SO_2), 127.9 (phenyl *C*), 127.7 (phenyl *C*H), 127.1 (indole *C*-C-NH), 123.2 (indole *C*H-NH), 122.1 (indole *C*H-CH-C-C), 119.6 (indole *C*H-C-C), 118.7 (indole *C*H-CH-C), 111.6 (indole *C*H-C-NH), 109.9 (indole *C*-CH_2), 82.3 (*C*(CH_3)$_3$), 66.8 (*C*H_2-phenyl), 53.9 (*C*H-CH_2-indole), 52.5 (*C*H-COO), 45.5 (*C*H_2-NSO_2), 41.8 (*C*H-CONH), 40.8 (*C*H_2-CH_2-NSO_2), 31.7

(<u>C</u>H_2-NHCbz), 29.8, 28.9, 27.8 (<u>C</u>H_2-CH, <u>C</u>H_2-CH_2-NHCbz and <u>C</u>H_2-indole), 27.9 (C(<u>C</u>H_3)$_3$), 21.5 (<u>C</u>H_2-CH_2-CH) and 21.0 (phenyl-<u>C</u>H_3); HR ESI *m/*z ($C_{42}H_{53}N_5NaO_8S$ requires 810.3493) found 810.3507.

2.39, (*S*)-*tert*-Butyl 2-((*R*)-2-(1-benzoylpiperidine-4-carboxamido)-3-(1*H*-indol-3-yl)propanamido)-6-(benzyloxycarbonylamino)hexanoate

Acid **2.12** (1.16 g, 4.98 mmol) was dissolved in dichloromethane (30 mL) and cooled to 0 ºC. HATU (1.93 g, 5.06 mmol) was added and the reaction was stirred for 10 minutes. Amine **2.31** (5 mmol) in dichloromethane (20 mL) and triethylamine (2.1 mL, 15 mmol) were added and the reaction stirred for 48 hours. The dichloromethane was removed *in vacuo*, the product dissolved in ethyl acetate was washed with a pH 2 buffer (3 × 50 mL) and ½ saturated $NaHCO_3$ (3 × 50 mL). The organic layer was dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate:MeOH 80:20:0 to 0:90:10) to give compound **2.39** as a beige solid (1.67 g, 55 %); *mp* 132-150 ºC (decomposed); υ_{max}/cm^{-1} 3285 (N-H), 2933 (saturated C-H), 1703 (ester C=O) and 1644 (amide C=O); δ_H (400 MHz, $CDCl_3$) 9.36 (br.s, 1H, N<u>H</u> indole), 7.74 (d, 1H, *J* 7.5, C<u>H</u>-C-C), 7.39-7.29 (m, 5H, phenyl), 7.15 (t, 1H, *J* 7.5, C<u>H</u>-CH-C), 7.10 (t, 1H, *J* 7.5, C<u>H</u>-CH-C-C), 6.96 (d, 1H, *J* 2, C<u>H</u>-NH), 6.57-6.51 (m, 1H, NH), 6.13, 6.00 (d, 1H, *J* 8, NH rotamers), 5.13 (s, 2H, C<u>H</u>$_2$-phenyl), 5.07 (t, 1H, *J* 6, N<u>H</u>Cbz), 4.83-4.78 (m, 1H, C<u>H</u>-CH_2-indole), 4.63 (br.s, 1H, C<u>H</u>$_2$-N equatorial), 4.40, 4.30 (q, 1H, *J* 5, C<u>H</u>-COO), 3.74 (br.s, 1H, C<u>H</u>$_2$-N equatorial), 3.30 (dd, 1H, *J* 4.5, 11.5, C<u>H</u>$_2$-indole), 3.13-3.02 (m, 2H, CH_2-N axial), 3.00-2.79 (m, 3H, C<u>H</u>$_2$-indole and C<u>H</u>$_2$-NHCbz), 2.34 (tt, 1H, *J* 1, 4.5, C<u>H</u>-CONH), 1.87-1.60 (m, 4H, C<u>H</u>$_2$-CH_2-N and C<u>H</u>$_2$-CH), 1.51-1.42 (m, 1H, C<u>H</u>$_2$-CH_2-NH), 1.36-1.29 (m, 1H, C<u>H</u>$_2$-CH_2-NH), 1.35 (s, 9H, C(C<u>H</u>$_3$)$_3$), 0.85-0.78 (m, 1H, C<u>H</u>$_2$-CH_2-CH) and 0.74-0.67 (m, 1H, C<u>H</u>$_2$-CH_2-

CH); δ_C (400 MHz, $CDCl_3$) 173.4 (*C*=O ester), 171.6, 171.2, 168.2 (*C*=O amide), 156.1 (*C*=O carbamate), 137.2 (indole *C*-NH), 136.3 (phenyl *C*), 135.9 (*C*), 129.3 (phenyl *C*H), 128.4 (phenyl *C*H), 128.3 (phenyl *C*H), 127.7 (phenyl *C*H), 127.4 (indole *C*-C-NH), 126.6 (phenyl *C*H), 123.6 (indole *C*H-NH), 120.7 (indole *C*H-CH-C), 118.5 (indole *C*H-CH-C-C), 118.1 (indole *C*H-C-C), 111.2 (indole *C*H-C-NH) and 109.9 (indole *C*-CH_2), 80.5 (*C*$(CH_3)_3$), 65.1 (*C*H_2-phenyl), 52.9 (*C*H-CH_2-indole), 52.6 (C*H*-COO), 41.3 (*C*H-CONH), 40.1 (*C*H_2-NCO), 39.9 (*C*H_2-CH_2-NCO), 30.7 (*C*H_2-NHCbz), 28.9, 28.4, 28.3 (*C*H_2-CH_2-NHCbz, *C*H_2-CH and *C*H_2-indole), 27.6 (C(*C*$H_3)_3$) and 22.4 (*C*H_2-CH_2-CH); ESI *m/*z 100 %, 760.6 (MNa^+).

2.40, (*S*)-*tert*-Butyl 6-(benzyloxycarbonylamino)-2-((*R*)-2-biphenyl-4-ylcarboxamido-3-(1*H*-indol-3-yl)propanamido)hexanoate

4-Biphenylcarboxylic acid (0.72 g, 3.6 mmol) was dissolved in dichloromethane (80 mL) and cooled to 0 ºC. HATU (1.39 g, 3.66 mmol) was added and the reaction was stirred for 10 minutes. Amine **2.31** (3.5 mmol) in dichloromethane and triethylamine (1.50 mL, 10.7 mmol) were added to the reaction. The reaction was stirred for 48 hours. The dichloromethane was removed *in vacuo*, the product dissolved in ethyl acetate was washed with a pH 2 buffer (3 × 50 mL) and ½ saturated $NaHCO_3$ (3 × 50 mL). The organic layer was dried over Na_2CO_3 and reduced *in vacuo*. The product was purified by silica column chromatography petroleum ether:ethyl acetate:MeOH (80:20:0 to 0:90:10) to give compound **2.40** as a beige solid (0.31g, 12 %); *mp* 172-174 ºC (decomposed); $[\alpha]^{25}_D$ (c = 0.5, MeOH) -11.54; υ_{max}/cm^{-1}: 3325 (N-H), 1708 (C=O ester/carbamate), 1665 (C=O amide) and 1530 (aromatic); δ_H (400 MHz, d_6-DMSO) 10.79 (d, 1H, N*H* indole), 8.55 (d, 1H, *J* 9.5, N*H*-CO-biphenyl), 8.39 (d, 1H, *J* 7, *N*H-CH-COO), 7.94 (d, 2H, *J* 8,biphenyl C*H*-C-CO), 7.74 (t, 5H, *J* 8.5, indole C*H*-C-C, biphenyl C*H*-C-phenyl, C*H*-C-aryl), 7.50 (t, 2H, *J* 8, biphenyl C*H*-

CH-C-aryl), 7.42 (d, 1H, *J* 7, biphenyl C*H*-CHCH-C-aryl), 7.37-7.30 (m, 6H, indole C*H*-C-NH and phenyl), 7.27-7.30 (m, 2H, N*H*Cbz & indole C*H*-NH), 7.07 (t, 1H, *J* 7.5, indole C*H*-CH-C), 6.99 (t, 1H, *J* 7.5, indole C*H*-CH-C-C), 5.00 (s, 2H, CH_2-phenyl), 4.84 (td, 1H, *J* 10, 5, C*H*-CH_2-indole), 4.16-4.10 (m, 1H, C*H*-COO), 3.27-3.13 (m, 2H, CH_2-indole), 2.98 (q, 2H, *J* 7, CH_2-NHCbz), 1.74-1.53 (m, 2H, CH_2-CH), 1.41 (s, 9H, C(CH_3)$_3$), 1.45-1.32 (m, 2H, CH_2-CH_2-NH) and 1.30-1.20 (m, 2H, (CH_2-CH_2-CH); δ_C (100 MHz, d_6-DMSO) 171.8 and 171.2 (ester and amide C=O), 165.6 (C=O biphenyl), 156.1 (C=O carbamate), 142.3 (biphenyl *C*-phenyl), 139.1 (biphenyl *C*-aryl), 137.2 (biphenyl *C*-CO), 136.1 (indole *C*-NH), 132.9 (phenyl *C*), 129.0 (biphenyl *C*H-CH-C-aryl), 128.3 (biphenyl *C*H-C-CO), 128.1, 128.0 (phenyl CH), 127.7 (indole *C*-C-NH), 127.3 (biphenyl *C*H-C-aryl), 127.0 (aryl *C*H-C-phenyl), 126.8 (biphenyl *C*H-CH-CH-C-aryl), 126.3 (phenyl *C*H), 123.7 (indole *C*H-NH), 120.8 (indole *C*H-CH-C-C), 118.6 (indole *C*H-C-C), 118.1 (indole *C*H-CH-C), 111.2 (indole *C*H-C-NH), 110.4 (indole *C*-CH_2), 80.5 (*C*(CH_3)$_3$), 65.1 (*C*H_2-phenyl), 54.0 (*C*H-CH_2-indole), 52.6 (*C*H-COO), 40.1 (*C*H_2-NHCbz), 30.8 (*C*H_2-CH), 28.9 (*C*H_2-indole), 27.9 (*C*H_2-CH_2-NHCbz), 27.6 (C(*C*H_3)) and 22.5 (*C*H_2-CH_2-CH); HR ESI *m/*z ($C_{42}H_{46}N_4NaO_6$ requires 725.3363) found 725.3319.

2.41, (*R*)-*N*-(1-(4-Aminobutylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)-1-tosylpiperidine-4-carboxamide

Carbamate **2.34** (0.21 g, 0.31 mmol) was dissolved in MeOH (2 mL). Charcoal activated palladium (0.08 g, catalytic) was added and the reaction was stirred under hydrogen for 24 hours. The reaction was filtered through celite and the MeOH removed *in vacuo* to give compound **2.41** as a colourless oil (0.14 g, 84 %); δ_H (400 MHz, $CDCl_3$) 9.10 (br.s, 1H, N*H* indole), 7.60 (d, 1H, *J* 8.5, indole C*H*-C-C), 7.58 (d, 2H, *J* 8.5, H), 7.35-7.27 (m, 3H, indole C*H*-C-NH and C*H*-CCH_3), 7.12 (t, 1H, *J*

8, indole C*H*-CH-C), 7.03 (t, 1H, *J* 7.5, indole C*H*-CH-C-C), 6.98 (s, 1H, indole C*H*-NH), 6.90 (br.s, 1H, N*H*-CH_2), 6.71 (br.d, 1H, *J* 7, N*H*-CH), 4.64 (q, 1H, *J* 7, C*H*-CH_2-indole), 3.72-3.62 (m, 2H, equatorial CH_2-N), 3.18 (dd, 1H, *J* 14, 5, CH_2-indole), 3.12-2.87 (m, 4H, CH_2-indole, CH_2-NHCO and CH_2-NH_2), 2.54-2.46 (m, 1H, CH_2-NHCO or CH_2-NH_2), 2.41 (s, 3H, CH_3), 2.20 (br.t, 2H, *J* 10, CH_2-N axial), 2.02-1.90 (m, 1H, C*H*-CCO), 1.81-1.60 (m, 4H, CH_2-CH_2N) and 1.29-1.11 (m, 4H, CH_2-CH_2-NH and CH_2-CH_2-NH_2); δ_C (100 MHz, $CDCl_3$) 173.7, 171.3 (*C*=O), 143.7 (*C*-CH_3), 136.3 (indole *C*-NH), 133.0 (*C*-SO_2), 129.7 (*C*H-CCH_3), 127.7 (*C*H-CSO_2), 127.5 (indole *C*-C-NH), 123.3 (indole *C*H-NH), 122.1 (indole *C*H-CH-C-C), 119.5 (indole *C*H-C-C), 118.8 (indole *C*H-CH-C), 111.4 (indole *C*H-C-NH), 110.6 (indole *C*-CH_2), 54.1 (*C*H-CH_2-indole), 45.5 (*C*H_2-NSO_2), 41.8 (*C*H-CH_2CH_2N), 40.9, 39.1 (*C*H_2-NH_2 and *C*H_2-NHCO), 29.0, 28.1, 27.9 (*C*H_2-CH_2NH_2, *C*H_2-CH_2N and *C*H_2-indole), 26.5 (*C*H_2-CH_2NH) and 21.5 (CH_3); ESI *m/*z 100 %, 540.30 (MH^+).

2.42, (*R*)-*N*-(1-(5-Aminopentylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)-1-tosylpiperidine-4-carboxamide

Carbamate **2.36** (0.10 g, 0.15 mmol) was dissolved in MeOH (1 mL) and THF (1 mL). Palladium activated charcoal (0.02 g, catalytic) was added and the reaction stirred under hydrogen for 24 hours. The reaction was filtered through celite and the organic solvent was removed *in vacuo* to give the product compound **2.42** as a colourless oil (0.069 g, 83 %); δ_H (400 MHz, MeOD) 7.65 (d, 2H, *J* 8, C*H*-CH_3), 7.60 (d, 1H, *J* 7.5, indole C*H*-C-C), 7.44 (d, 2H, *J* 8, C*H*-CSO_2), 7.35 (d, 1H, *J* 7.5, indole C*H*-C-NH), 7.11 (t, 1H, *J* 8, indole C*H*-CH-C), 7.08 (s, 1H, indole C*H*-NH), 7.02 (t, 1H, *J* 8, indole C*H*-CH-C-C), 4.60 (t, 1H, *J* 7.5, C*H*-CH_2-indole), 3.62 (br.d, 1H, *J* 11.5, equatorial CH_2-N), 3.61 (br.d, 1H, *J* 11.5, equatorial CH_2-N), 3.21 (dd,

1H, *J* 15, 7, C*H*$_2$-indole), 3.17-2.98 (m, 3H, C*H*$_2$-indole, C*H*$_2$-NHCO), 2.56 (t, 1H, *J* 7, C*H*$_2$-NH$_2$), 2.46 (s, 4H, C*H*$_3$ and C*H*$_2$-NH$_2$), 2.30 (qd, 2H, *J* 11, 2, C*H*$_2$-CH$_2$N) 2.16 (tt, 1H, *J* 11, 4, C*H*-CH$_2$CH$_2$N), 1.79 (br.d, 1H, *J* 15, C*H*$_2$-CH$_2$N), 1.74-1.50 (m, 3H, C*H*$_2$-CH$_2$N), 1.45-1.25 (m, 4H, C*H*$_2$-CH$_2$NHCO and C*H*$_2$-CH$_2$NH$_2$) and 1.19-1.10 (m, 2H, C*H*$_2$-CH$_2$CH$_2$NH$_2$).

2.01, (*S*)-*tert*-Butyl 2-((*R*)-3-(1*H*-indol-3-yl)-2-(1-(*meta*-tolylsulfonyl)piperidine-4-carboxamido)propanamido)-6-aminohexanoate

Carbamate **2.37** (1.6 g, 2.0 mmol) was dissolved in MeOH (10 mL). Palladium activated charcoal (0.1 g, catalytic) was added and the reaction stirred under hydrogen for 48 hours. The reaction was filtered through celite and the organic solvents removed *in vacuo* to give the product compound **2.01** as a beige solid (0.33 g, 13 %); *mp*; 103-104 °C; $[\alpha]^{24}_{D}$ (c = 0.245, $CHCl_3$) -5.41; ; υ_{max}/cm^{-1}: 3302 (N-H), 2955 (saturated C-H), 1702 (ester C=O), 1664 (amide C=O), 1337, 1153 (SO_2) and 1251 (ester C-O); δ_H (400 MHz, MeOD) 7.62-7.55 (m, 3H, C*H*-CH-CHCCH$_3$, C*H*-CHCCH$_3$, C*H*CCH$_3$), 7.54-7.52 (m, 2H, indole C*H*-C-C and C*H*-CSO$_2$), 7.36 (d, 1H, *J* 8, indole C*H*-C-NH), 7.50 (t, 1H, *J* 7.5, indole C*H*-CH-C), 7.11 (s, 1H, indole C*H*-NH), 7.04 (t, 1H, *J* 7.5, indole C*H*-CH-C-C), 4.73 (t, 1H, *J* 7.5, C*H*-CH$_2$-indole), 4.10 (dd, 1H, *J* 9, 5, C*H*-COO), 3.34 (d, 1H, *J* 12, equatorial C*H*$_2$-N), 3.68 (d, 1H, *J* 12, equatorial C*H*$_2$-N), 3.23 (dd, 1H, *J* 14, 8, C*H*$_2$-indole), 3.09 (dd, 1H, *J* 14, 7, C*H*$_2$-indole), 2.56 (t, 2H, *J* 7.5, C*H*$_2$-NH$_2$), 2.45 (s, 3H, C*H*$_3$), 2.34 (qd, 2H, *J* 11.5, 3.5, axial C*H*$_2$-N), 2.20 (tt, 1H, *J* 11.5, 4, C*H*-CH$_2$CH$_2$), 1.83 (d, 1H, *J* 14, C*H*$_2$-CH$_2$N), 1.78-1.55 (m, 5H, C*H*$_2$-CH$_2$N and C*H*$_2$-CH-COO^{t}Bu), 1.46 (s, 9H, C(C*H*$_3$)$_3$), 1.36 (quin., 2H, C*H*$_2$-CH$_2$NH$_2$) and 1.00 (quin., 2H, C*H*$_2$-CH$_2$CH); δ_C (100 MHz, MeOD) 176.6, 173.8, 172.7 (*C*=O ester and amide), 140.9 (*C*-CH$_3$), 138.0 (*C*-SO$_2$), 137.2 (indole *C*-NH), 135.0 (*C*H-CCH$_3$), 130.3 (*C*H-CHCCH$_3$), 129.1 (*C*H-CSO$_2$),

129.0 (indole *C*-C-NH), 126.0 (*C*H-CHCHCCH$_3$), 124.8 (indole *C*H-NH), 122.6 (indole *C*H-CH-C-C), 120.0 (indole *C*H-C-C), 119.6 (indole *C*H-CH-C), 112.5 (indole *C*H-C-NH), 111.1 (indole *C*-CH$_2$), 82.8 (*C*(CH$_3$)$_3$), 55.5 (*C*H-CH$_2$-indole), 54.6 (*C*H-COO), 46.9 (*C*H$_2$-N), 42.6 (*C*H-CH$_2$CH$_2$N), 42.1 (*C*H$_2$-NH$_2$), 32.8, 32.2, 31.4, 29.2 (*C*H$_2$-indole, *C*H$_2$-CH$_2$N, *C*H$_2$-CH$_2$NH$_2$, *C*H$_2$-CHCOO), 28.4 (*C*H$_3$-aryl), 23.8 (*C*H$_2$-CH$_2$-CH-COO) and 21.6 (C(*C*H$_3$)$_3$); ESI *m/z* 100 %, 654.3 (MH$^+$), 7 %, 676.2 (MNa$^+$); HR ESI *m/*z ($C_{34}H_{48}N_5O_6SH^+$ requires 654.3320) found 654.3329.

2.43, (*S*)-*tert*-Butyl 2-((*R*)-3-(1*H*-indol-3-yl)-2-(1-tosylpiperidine-4-carboxamido) propanamido)-6-aminohexanoate

Carbamate **2.38** (1.55 g, 1.96 mmol) was dissolved in MeOH (10 mL) and THF (5 mL). Palladium activated charcoal (0.08 g, catalytic) was added and the reaction was stirred under hydrogen for 24 hours. The reaction was filtered through celite and reduced *in vacuo* to give compound **2.43** (0.11g, 99 %); *mp* 127-131 ºC (decomposed); υ_{max}/cm^{-1}: 3385 (N-H), 2926 (saturated C-H), 172 (ester C=O), 1634 (amide C=O), 1538 (NH$_2$) and 1331, 1145 (SO$_2$); δ_H (400 MHz, CDCl3) 9.26 (s, 1H, N*H* indole), 7.58 (d, 3H, *J* 8, indole C*H*-C-C C*H*-CSO$_2$), 7.30 (t, 3H, *J* 8, indole C*H*-C-NH and C*H*-CCH$_3$), 7.11 (t, 1H, *J* 7, indole C*H*-CH-C), 7.03 (t, 1H, *J* 7.5, indole C*H*-CH-C-C), 6.99 (s, 1H, indole C*H*-NH), 6.53 (d, 1H, *J* 8, N*H*-CHCH$_2$-indole), 6.49 (d, 1H, *J* 7 N*H*-CHCOO), 4.69 (q, 1H, *J* 8, C*H*-CH$_2$-indole), 4.24 (br.q, 1H, *J* 7, C*H*-COO), 3.62-3.73 (m, 2H, equatorial C*H*$_2$-N), 3.20 (dd, 1H, *J* 15, 7, C*H*$_2$-indole), 3.10 (dd, 1H, *J* 14, 8, CH$_2$-indole), 2.68-2.78 (br.s, 2H, C*H*$_2$-NH$_2$), 2.50-2.62 (br.d, 2H, axial C*H*$_2$-N), 2.40 (s, 3H, C*H*$_3$), 2.15-2.26 (br.t, 2H, equatorial C*H*$_2$-CH$_2$N), 1.89-1.99 (m, 1H, C*H*-CH$_2$CH$_2$N), 1.61-1.79 (m, 4H, axial C*H*$_2$-CH$_2$N and C*H*$_2$-CHCOO), 1.37 (s, 9H, C(C*H*$_3$)$_3$), 1.41-1.34 (m, 2H, C*H*$_2$-CH$_2$NH$_2$) and 0.87-0.99 (m, 2H, C*H*$_2$-CH$_2$CHCOO); δ_C (100 MHz, CDCl$_3$) 173.9, 171.2, 171.1 (*C*=O

ester and amide), 143.7 (<u>C</u>-CH_3), 136.4 (indole <u>C</u>-NH), 133.0 (<u>C</u>-SO_2), 129.7 (<u>C</u>H-CCH_3), 127.7 (<u>C</u>H-CSO_2), 127.4 (indole <u>C</u>-C-NH), 123.4 (indole <u>C</u>H-NH), 122.1 (indole <u>C</u>H-CH-C), 119.5 (indole <u>C</u>H-CH-C-C), 118.6 (indole <u>C</u>H-C-C), 111.5 (indole <u>C</u>H-C-NH), 110.1 (indole <u>C</u>-CH_2), 82.2 (C(<u>C</u>H_3)$_3$), 54.1 (<u>C</u>H-CH_2-indole), 52.8 (<u>C</u>H-COO), 45.5 (<u>C</u>H_2-NSO_2), 41.8 (<u>C</u>H-CH_2CH_2N) 41.0 (<u>C</u>H_2-CH_2N), 31.5 (<u>C</u>H_2-NH_2), 31.1, 28.0, 27.85, (<u>C</u>H_2-CH_2NH_2, <u>C</u>H_2-CHCOO and <u>C</u>H_2-indole), 27.7 (<u>C</u>H_2-CH_2CHCOO), 27.9 ((C(<u>C</u>H_3)$_3$) and 21.5 (<u>C</u>H_3); ESI *m/*z 100 %, 654.5 (MH^+).

2.44, (*S*)-*tert*-Butyl 6-amino-2-((*R*)-2-(1-benzoylpiperidine-4-carboxamido)-3-(1*H*-indol-3-yl)propanamido)hexanoate

Carbamate **2.39** (1.57 g, 2.13 mmol) was dissolved in MeOH (20 mL). Palladium activated charcol (0.08 g, catalytic) was added and the reaction was stirred under hydrogen for 24 hours. The reaction was filtered through celite and the organic solvents removed *in vacuo* to give compound **2.44** as a white solid (0.95 g, 63 %); *mp* 103-105 ºC; υ_{max}/cm^{-1}: 3279 (N-H), 2927 (saturated C-H), 1732 (ester C=O), 1645 (amide C=O) and 1615 (NH_2); HR ESI *m/*z ($C_{34}H_{45}N_5O_5$ require 604.3493) found 604.3500.

2.45, (*S*)-*tert*-Butyl 6-amino-2-((*R*)-2-biphenyl-4-ylcarboxamido-3-(1*H*-indol-3-yl)propanamido)hexanoate

Carbamate **2.40** (0.52 g, 0.74 mmol) was dissolved in THF (15 mL). Palladium activated charcoal (0.3 g, catalytic) was added and the reaction stirred under hydrogen for 24 hours. The palladium was removed through filtration and the organic solvent was removed *in vacuo* to give compound **2.45** as a brown oil (0.19 g, 46 %); υ_{max}/cm^{-1}: 3272 (N-H), 2929 (saturated C-H), 1724 (C=O ester), 1638 (C=O amide) and 1528 (aromatic); δ_H (400 MHz, MeOD) 7.87 (d, 2H, *J* 8.5, C<u>*H*</u>-CCO),

7.70 (d, 1H, *J* 8, indole C*H*-C-C), 7.63 (d, 2H, *J* 8.5, C*H*-C-aryl or C*H*-C-phenyl), 7.60 (d, 2H, *J* 8.5, C*H*-C-aryl or C*H*-C-phenyl), 7.43 (t, 2H, *J* 7.5, C*H*-CH-C-aryl), 7.40-7.33 (m, 4H, indole C*H*-C-NH and C*H*-CHCHC-aryl), 7.22 (s, 1H, indole C*H*-NH), 7.13 (t, 1H, *J* 7.5, indole C*H*-CH-C), 7.06 (t, 1H, *J* 7.5, indole C*H*-CH-C-C), 5.02 (t, 1H, *J* 7.5, C*H*-CH_2-indole), 4.19 (dd, 1H, *J* 5, 9, C*H*-COO), 3.45 (dd, 1H, *J* 14, 8, C*H*$_2$-indole), 3.38-3.31 (m, 1H, C*H*$_2$-indole obscured by MeOD), 2.60 (t, 2H, *J 7*, C*H*$_2$-NH_2), 1.67-1.57 (m, 1H, (C*H*$_2$-CH), 1.53-1.34 (m, 3H, C*H*$_2$-CH & C*H*$_2$-CH_2NH_2), 1.42 (s, 9H, C(C*H*$_3$)$_3$) and 1.00 (quin., 2H, (C*H*$_2$-CH_2CH); δ_C (100 MHz, MeOD) 174.1, 172.7, 169.5 (*C*=O ester and amide), 145.8, 139.1, 137.2 (*C*-aryl), 133.9 (indole *C*-NH), 130.1 (C*H*-CH-C-aryl), 128.3 (*C*H-C-CO), 129.0 (indole *C*-C-NH), 128.2 (*C*H-C-aryl), 127.80 (*C*H-C-phenyl), 126.2 (*C*H-CHCHC-aryl), 124.8 (indole *C*H-NH), 122.6 (indole *C*H-CH-C-C), 120.0 (indole *C*H-C-C), 119.6 (indole *C*H-CH-C), 112.5 (indole *C*H-C-NH), 111.1 (indole *C*-CH_2), 82.9 (*C*(CH_3)$_3$), 56.5 (*C*H-CH_2-indole), 54.6 (*C*H-COO), 41.4 (*C*H_2-NH_2), 32.0 (*C*H_2-CH-COO), 31.1 (*C*H_2-indole), 29.4 (*C*H_2-CH_2NH_2), 28.3 (C(*C*H_3)) and 23.7 (*C*H_2-CH_2CH); ESI *m/*z 100 %, 569.2 (MH^+) and 11 %, 489.3 (M-$C_5H_8O_2$); HR ESI *m/*z ($C_{34}H_{40}N_4O_4H^+$ requires 569.3122) found 569.3147.

2.46, (D)-Tryptophan methyl ester hydrogen chloride

D-Tryptophan (10 g, 49 mmol) was added to MeOH (100 mL) to form a suspension. Thionyl chloride (5.0 mL, 69 mmol) was slowly added to MeOH (50 mL) which was added to the suspension. The reaction was heated under reflux conditions, under nitrogen. After 3 hours the reaction was cooled and reduced *in vacuo* to 75 mL, dichloromethane was added and the reaction cooled to 0-5 ºC and stirred for 30 minutes. The resulting precipitate was filtered and washed with dichloromethane to

give compound **2.46** as a white solid (4.91 g, 46 %); δ_H (400 MHz, D_2O) 7.56 (d, 1H, *J* 8, indole C*H*-C-C), 7.48 (d, 1H, *J* 7.5, indole C*H*-C-NH), 7.25 (s, 1H, indole C*H*-NH), 7.22 (t, 1H, *J* 8, indole C*H*-CH-C), 7.14 (t, 1H, *J* 8, indole C*H*-CH-C-C), 4.40 (t, 1H, *J* 6.5, C*H*-CH_2-indole), 3.75 (s, 3H, C*H*$_3$), 3.45 (dd, 1H, *J* 14, 7, C*H*$_2$-indole) and 3.39 (dd, 1H, *J* 14, 7, C*H*$_2$-indole); δ_C (100 MHz, D_2O) 170.4 (*C*=O), 136.2 (indole *C*-NH), 126.3 (indole *C*-C-NH), 125.4 (indole *C*H-NH), 122.2 (indole *C*H-CH-C-C), 119.6 (indole *C*H-C-C), 118.0 (indole *C*H-CH-C), 112.0 (indole *C*H-C-NH), 105.9 (indole *C*-CH_2), 53.3 (*C*H-CH_2-indole and *C*H_3) and 25.6 (*C*H_2-indole).

2.47, 4-(2-Keto-1-benzimidazolinyl)piperidine-(D)-Trp-OMe

Amine **2.46** (2.55 g, 10.0 mmol), *N*,*N*-diisopropylethylamine (5.0 mL, 30 mmol) and disuccinidyl carbonate (2.56 g, 10.0 mmol) were dissolved in dichloromethane (50 mL) and stirred for 30 minutes. 4-(2-Keto-1-benzimidazolinyl) piperidine (2.17 g, 9.98 mmol) was added to the reaction which was stirred overnight. The dichloromethane was removed *in vacuo*, the product dissolved in ethyl acetate and washed with a pH 2 buffer (3 × 20 mL) and ½ saturated $NaHCO_3$ (3 × 20 mL). The organic layer was dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (ethyl acetate:MeOH, 100:0 to 80:20) to give compound **2.47** as a white solid (0.83 g, 39 %); *mp* 134-136 º C; $[\alpha]^{20}_D$ (c = 0.526, MeOH) 12.45; υ_{max}/cm^{-1}: 3269 (N-H), 2949 (saturated C-H), 1688, 1622 (C=O) and 1366 (C-O); δ_H (500 MHz, $CDCl_3$) 9.37 (s, 1H, N*H*-indole), 8.31 (br.s, 1H, N*H*-C-CH), 7.59 (d, 1H, *J* 8, indole C*H*-C-C), 7.35 (d, 1H, *J* 8.5, indole C*H*-C-NH), 7.17 (t, 1H, *J* 7.5, indole C*H*-CH-C), 7.13-7.00 (m, 6H, indole C*H*-NH, indole C*H*-CH-C-C and aryl), 5.1 (d, 1H, *J* 7, N*H*-$CHCH_2$-indole), 4.88 (q, 1H, *J* 6, C*H*-CH_2-indole),

4.43 (tt, 1H, *J* 12.5, 4, C*H*-NCO) 4.06 (br.d, 1H, *J* 13.5, equatorial C*H*$_2$-N), 4.00 (br.d, 1H, *J* 13.5, equatorial C*H*$_2$-N), 3.74 (s, 3H, C*H*$_3$), 3.39 (dd, 1H, *J* 15, 5, C*H*$_2$-indole), 3.33 (dd, 1H, *J* 14.5, 5, C*H*$_2$-indole), 2.87 (td, 1H, *J* 13,2, axial C*H*$_2$-N), 2.82 (td, 1H, *J* 13,2, axial C*H*$_2$-N), 2.32 (qd, 1H, *J* 12.5, 4.5, C*H*$_2$-CH$_2$N), 2.27 (qd, 1H, *J* 12.5, 4.5, C*H*$_2$-CH$_2$N) and 1.76 (br.d, 2H, *J* 13, equatorial C*H*$_2$-CH$_2$N); δ_C (125 MHz, $CDCl_3$) 173.8 (*C*=O ester), 156.9, 155.1 (*C*=O urea), 136.4 (indole *C*-NH), 128.2, 127.7 (*C*-NCO and *C*-NHCO), 127.5 (indole *C*-C-NH), 123.2 (indole *C*H-NH), 122.1 (*C*H-CHCN or *C*H-CH-C-NHCO), 121.8 (*C*H-CHCN or *C*H-CH-C-NHCO), 121.5 (indole *C*H-CH-C-C), 119.5 (indole *C*H-C-C), 118.4 (indole *C*H-CH-C), 111.6 (indole *C*H-C-NH), 109.9 (*C*H-CN or *C*H-CNHCO), 109.8 (C8) 109.4 (*C*H-CN or *C*H-CNHCO), 54.8 (*C*H-CH$_2$-indole), 52.3 (O-*C*H$_3$), 50.6 (*C*H-N-CO), 44.5 (*C*H$_2$-N), 29.0 (*C*H$_2$-CH$_2$N) and 27.8 (*C*H$_2$-indole); ESI *m/*z 100 %, 484.2 (MNa^+) and 31 % (MH^+).

2.48, (2*R*,2'*R*)-dimethyl 2,2'-carbonylbis(azanediyl)bis(3-(1*H*-indol-3-yl)propanoate)

The other product isolated in the synthesis of compound **2.47** was compound **2.48**; δ_H (400 MHz, $CDCl_3$) 8.46 (br.s, 2H, indole N*H*), 7.42 (d, 2H, *J* 8, indole C*H*-C-C), 7.25 (d, 2H, *J* 8, indole C*H*-C-NH), 7.12 (t, 2H, *J* 8, indole C*H*-CH-C-NH), 7.04 (t, 2H, *J* 8, indole C*H*-CH-C-C), 6.70 (s, 2H, indole C*H*-NH), 5.86 (d, 2H, N*H*-CO), 4.82 (dt, 2H, *J* 8, 5.5, C*H*-CH$_2$-indole), 3.39 (s, 6H, O-C*H*$_3$) and 2.97 (d, 4H, C*H*$_2$-indole); δ_C (100 MHz, $CDCl_3$) 174.1 (*C*=O ester), 157.3 (*C*=O urea), 136.0 (indole *C*-NH), 127.4 (indole *C*-C-NH), 123.5 (indole *C*H-NH), 121.9 (indole *C*H-CH-C-C), 119.4 (indole *C*H-CH-C-NH), 118.4 (indole *C*H-C-C), 111.4 (indole *C*H-CH-NH), 109.3 (indole *C*-CH$_2$), 53.1, 52.3 (*C*H-CH$_2$-indole) and 28.1 (*C*H$_2$-indole).

2.49, 4-(2-Keto-1-benzimidazolinyl)piperidine-(D)-Trp-OH

Ester **2.47** (0.60 g, 1.3 mmol) was dissolved in THF (20 mL). LiOH (0.13 g, 5.4 mmol) was dissolved in D_2O (20 mL), ethanol (5 mL) and added to the THF and stirred for 4 hours at room temperature. The reaction was acidified with HCl to pH 2, extracted with ethyl acetate and washed with NaCl (3 × 15 mL). The organic layer was dried over Na_2SO_4 and reduced *in vacuo* to give compound **2.49** as a beige solid (0.52 g, 93 %) *mp* 187-188 °C; $[\alpha]^{22}_D$ (c = 1, MeOH) 0.7; δ_H; υ_{max}/cm^{-1}: 3316 (N-H), 3060 (O-H carboxylic acid), 2932 (saturated C-H) and 1656, 1625 (C=O urea); δ_H (400 MHz, MeOD) 7.66 (d, 1H, *J* 8, indole C*H*-C-C), 7.35 (d, 1H, *J* 8, indole C*H*-C-NH), 7.18 (s, 1H, indole C*H*-NH), 7.16-7.03 (m, 6H, C*H*-NH, indole C*H*-CH-C-C and aryl), 4.68 (dd, 1H, *J* 8, 5, C*H*-CH_2-indole), 4.46 (tt, 1H, *J* 12.5, 4, C*H*-NCO) 4.18-4.08 (m, 2H, equatorial CH_2-N), 3.44 (dd, 1H, *J* 14.5, 5, CH_2-indole), 3.27 (dd, 1H, *J* 14.5, 8, CH_2-indole), 2.91 (qd, 2H, *J* 13.5, 2, axial CH_2-N), 2.35 (qd, 1H, *J* 12.5 ,4.5, axiaCH_2-CHN l), 2.22 (qd, 1H, *J* 12.5, 4.5, axial CH_2-CHN), and 1.72 (tt, 2H, *J* 10, 2.5, equatorial CH_2-CH_2N); δ_C (100 MHz, MeOD) 176.6 (C=O acid), 159.6 (C=O urea), 156.2 (C=O urea), 138.1 (indole *C*-NH), 130.2, 129.6 (C-NCO and C-NHCO), 129.0 (indole *C*-C-NH), 124.5 (indole *C*H-NH), 122.5 (*C*H-CHCN or *C*H-CH-C-NHCO), 122.48 (*C*H-CHCN or *C*H-CH-C-NHCO), 122.4 (indole *C*H-CH-C-C), 119.9 (indole *C*H-C-C), 119.3 (indole *C*H-CH-C), 112.4 (indole *C*H-C-NH), 111.6 (indole *C*-CH_2), 110.6 (*C*H-CN or *C*H-CNHCO), 110.56 (*C*H-CN or *C*H-CNHCO), 56.5 (*C*H-CH_2-indole), 52.1 (*C*H-NCO), 45.0, 44.9 (*C*H_2-N), 30.9 (*C*H_2-CH_2N) and 30.0 (*C*H_2-indole); ESI *m/*z 100 %, 448.2 (MH^+) and 17 %, 470.1 (MNa^+).

2.50, 4-(2-Keto-1-benzimidazolinyl)piperidine-(D)-Trp-(L)-Lys(Z)-O^{t}Bu

Acid **2.49** (0.51 g, 1.1 mmol,) was dissolved in dichloromethane (20 mL) and cooled to 0 °C. HATU (0.44 g, 1.2 mmol) was added and the reaction was stirred for 10 minutes. Amine **2.21** (0.43 g, 1.2 mmol) and triethylamine (0.50 mL, 3.4 mmol) were added and the reaction was stirred for 24 hours. The dichloromethane was removed *in vacuo*, the product dissolved in ethyl acetate was washed with a pH 2 buffer (3 × 15 mL), 0.1 M HCl (3 × 15 mL) and ½ saturated $NaHCO_3$ (3 × 15 mL). The organic layer was dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate:MeOH 25:75:0 to 0:90:10) to give compound **2.50** as a white solid (0.22 g, 25 %); δ_H (400 MHz, MeOD) 7.67 (d, 1H, *J* 7.5, indole C*H*-C-C), 7.38-7.24 (m, 6H, indole C*H*-C-NH and phenyl), 7.16 (s, 1H, indole C*H*-NH), 7.12-7.00 (m, 6H, indole C*H*-CH-C, indole C*H*-CH-C-C and aryl), 5.06 (s, 2H, CH_2-phenyl), 4.70 (t, 1H, *J* 7.5, C*H*-CH_2-indole), 4.40 (tt, 1H, *J* 12.5, 4, C*H*-NCO), 4.23 (dd, 1H, *J* 8, 5, C*H*-COO), 4.17 (br.d, 1H, *J* 14, equatorial CH_2-N), 4.12-4.07 (m, 1H, equatorial CH_2-N), 3.34 (dd, 1H, *J* 14, 7, CH_2-indole), 3.21 (dd, 1H, *J* 14, 7.5, CH_2-indole), 3.07 (t, 2H, *J* 7, CH_2-NHCbz), 2.87 (br.t, 1H, *J* 13, axial CH_2-N), 2.82 (br.t, 1H, *J* 13, axial CH_2-N), 2.29 (qd, 1H, *J* 12.5 ,4.5, axial CH_2-CH-N), 2.15 (qd, 1H, *J* 12.5, 4, axial CH_2-CH-N), 1.72-1.91 (m, 3H, CH_2-CH_2NHCbz and CH_2-CHCOO), 1.59-1.50 (m, 1H, CH_2-CH_2NHCbz and CH_2-CH-COO), 1.47-1.36 (m, 2H, axial CH_2-CH-N), 1.45 (s, 9H, C(CH_3)$_3$) and 1.19-1.07 (m, 2H, CH_2-CH_2CHCOO); δ_C (100 MHz, MeOD) 175.1, 172.8 (*C*=O ester and amide), 159.0, 158.9, 156.2 (*C*=O urea and carbamate), 138.4 (indole *C*-NH), 138.0 (phenyl *C*), 130.3, 129.7 (*C*-NCO and *C*-NHCO), 129.5 (phenyl *C*H), 129.0 (indole *C*-C-NH), 128.99, 128.90 (phenyl *C*H), 124.7 (indole

*C*H-NH), 122.6, 122.4 (*C*H-CHCN and *C*H-CH-C-NHCO), 120.0, 119.9, 119.5 (indole *C*H-CH-C, *C*H-CH-C-C and *C*H-C-C), 112.5 (indole *C*H-C-NH), 111.4 (indole *C*-CH_2), 110.6, 110.5 (*C*H-CN and *C*H-CNHCO), 82.9 (*C*$(CH_3)_3$), 67.4 (*C*H_2-phenyl), 57.3 (*C*H-CH_2-indole), 54.5 (*C*H-COO), 52.1 (*C*H-NCO), 45.0, 44.9 (*C*H_2-N), 41.6 (*C*H_2-NHCbz), 32.3 (*C*H_2-CH-COO), 30.5 (*C*H_2-CH_2N), 29.5 (*C*H_2-indole), 28.4 (*C*H_2-CH_2NHCbz), 23.7 (C(*C*$H_3)_3$) and 21.0 (*C*H_2-CH_2CHCOO); ESI *m/*z 100 %, 766.4 (MH^+).

2.51, 4-(2-Keto-1-benzimidazolinyl)piperidine-(D)-Trp-(L)-Lys-O^{t}Bu

Carbamate **2.50** (0.22 g, 0.29 mmol) was dissolved in MeOH (5 mL). Palladium activated charcoal (0.02 g, catalytic) was added and the reaction stirred under hydrogen for 24 hours. The palladium was removed through filtration, and the MeOH was removed *in vacuo* to give compound **2.51** as a white solid (0.1 g, 55 %); δ_H (400 MHz, MeOD) 7.66 (d, 1H, *J* 8, indole C*H*-C-C), 7.35 (d, 1H, indole C*H*-C-NH), 7.17 (s, 1H, indole C*H*-NH), 7.11-7.01 (m, 6H, indole C*H*-CH-C, indole C*H*-CH-C-C and aryl), 4.68 (t, 1H, *J* 7, C*H*-CH_2-indole), 4.42 (tt, 1H, *J* 12.5, 4, C*H*-NCO), 4.21 (m, 1H,C*H*-COO), 4.12 (d, 1H, *J* 12.5, equatorial C*H*$_2$-N), 4.16 (d, 1H, *J* 12.5, equatorial C*H*$_2$-N), 3.38-3.30 (m, 1H, C*H*$_2$-indole - obscured by MeOD), 3.24-3.13 (m, 1H, C*H*$_2$-indole), 2.91 (t, 1H, *J* 10, axial C*H*$_2$-N), 2.86 (t, 1H, *J* 10, axial C*H*$_2$-N), 2.55 (t, 2H, *J* 7.5, C*H*$_2$-NH_2), 2.31 (qd, 1H, *J* 12.5 ,4.5, axial C*H*$_2$-CH-N), 2.17 (qd, 1H, *J* 12.5, 4, axial C*H*$_2$-CH-N), 1.75-1.62 (br.t, 3H, *J* 12.5, C*H*$_2$-CHCOO and equatorial C*H*$_2$-CH_2N), 1.60-1.50 (m, 1H, C*H*$_2$-CHCOO), 1.48-1.36 (m, 2H, C*H*$_2$-CH_2NH_2), 1.46 (s, 9H, C(C*H*$_3)_3$) and 1.15-1.07 (m, 2H, C*H*$_2$-CH_2CHCOO); δ_C (100 MHz, MeOD) 175.2, 172.8 (*C*=O ester and amide), 159.0, 156.2 (*C*=O urea), 138.1 (indole *C*-NH), 130.3, 129.7 (*C*-NCO and *C*-NHCO), 129.0 (indole *C*-C-NH),

126.2 (indole <u>C</u>H-NH), 124.7, 122.6 (<u>C</u>H-CHCN and <u>C</u>H-CH-C-NHCO), 122.4, 120.0, 119.5 (indole <u>C</u>H-CH-C, <u>C</u>H-CH-C-C and <u>C</u>H-C-C), 112.5 (indole <u>C</u>H-C-NH), 111.4 (indole <u>C</u>-CH_2), 110.6, 110.5 (<u>C</u>H-CN and <u>C</u>H-CNHCO), 82.9 (<u>C</u>$(CH_3)_3$), 57.4 (<u>C</u>H-CH_2-indole), 54.6 (<u>C</u>H-COO), 52.1 (<u>C</u>H-NCO), 45.0, 44.9 (<u>C</u>H_2-N), 42.2 (<u>C</u>H_2-NH_2), 33.2 (<u>C</u>H_2-CH-COO), 32.3 (<u>C</u>H_2-CH_2N), 30.08 (<u>C</u>H_2-indole), 29.5 (<u>C</u>H_2-CH_2NH_2), 28.3 (C(<u>C</u>$H_3)_3$) and 23.8 (<u>C</u>H_2-CH_2-CH-COO); ESI *m/*z 100 %, 632.3 (MH^+); HR *m/*z ($C_{34}H_{45}N_7O_5H^+$ requires 632.555) found 632.3578.

6.2.2 Potential BSCIs - lactam containing compounds

2.52, 3-Amino-azepan-2-one

Acetyl chloride (118 mL, 1.65 mol) was carefully dissolved in MeOH (2 L). (L)-Lysine.HCl (200 g, 1.10 mol) was added and the reaction heated under reflux conditions over night. The organic solvents were removed *in vacuo* to give (L)-lys-methyl ester (239.6 g, 1.028 moles) as a white solid which was dissolved in MeOH (2 L). Sodium methoxide (470 mL, 2.06 mol) was added and the reaction was heated under reflux conditions and under nitrogen for 48 hours, then allowed to slowly cool for a further 48 hours. The NaCl precipitate was removed by filtration, dimethoxyethane was added and the resulting precipitate was removed by filtration. The organic solvents were then removed *in vacuo* to give compound **2.52** an oil which crystallised to a white solid when left to stand (60.60 g, 46 %); δ_H (400 MHz, D_2O) 4.24 (dd, 1H, *J* 10, 1.5, C<u>*H*</u>-CO), 3.27-3.11 (m, 2H, lactam C<u>*H*</u>$_2$-NH), 2.07-1.90 (m, 2H, C<u>*H*</u>$_2$-CH), 1.82 (dd, 1H, *J* 14, 4, equatorial lactam C<u>*H*</u>$_2$-CH_2NH), 1.75-1.63 (m, 2H, lactam C<u>*H*</u>$_2$-CH_2CH) and 1.36 (ddt, 1H, J 15, 11, 3, axial lactam C<u>*H*</u>$_2$-CH_2NH); δ_C (100 MHz, D_2O) 173.9 (C=O), 52.9 (<u>C</u>H), 41.2 (lactam <u>C</u>H_2-NH), 28.3

(lactam $\underline{C}H_2$-CH), 27.4 (lactam $\underline{C}H_2$-CH_2CH) and 26.9 (lactam $\underline{C}H_2$-CH_2NH); ESI *mz* 100 % (129.1).

2.53, (*S*)-3-Amino-tetrahydropyridin-2-one

Acetyl chloride (32.0 mL, 448 mmol) was dissolved in MeOH (150 mL). (L)-Ornithine.HCl (30.0 g, 178 mmol) was added and the reaction heated under reflux conditions for 3 hours. The reaction was then cooled to 0 °C and sodium methoxide (120 mL) was added until pH 9-10 was reached, the reaction was stirred for 2.5 hours. Diethyl ether (75 mL) was added and the precipitate removed by filtration, the organic solvents were then removed *in vacuo* to give compound **2.53** as a white oily solid which was not isolated and immediately dissolved in H_2O and reacted.

2.54, (*R*)-3-Amino-tetrahydropyridin-2-one

Acetyl chloride (0.70 mL, 9.8 mmol) was dissolved in MeOH (20 mL). (D)-Ornithine.HCl (0.720 g, 4.26 mmol) was added and the reaction heated under reflux conditions for 3 hours. The reaction was then cooled to 0 °C and sodium methoxide (1.5 mL) was added until pH 9-10 was reached, the reaction was stirred for 2.5 hours. On completion of the reaction the organic solvents were then removed *in vacuo* to give compound **2.54** as a white oily solid which was not isolated immediately dissolved in H_2O and reacted.

2.55, (*S*)-3-Amino-azepan-2-one hydropyroglutamate

Amine **2.52** (20.9 g, 163 mmol) was dissolved in dimethoxyethane (500 mL) and heated to 70 °C. (*S*)-(−)-2-Pyrrolidone-5-carboxylic acid (21.70 g, 168.10 mmol) was added slowly and the reaction was heated for 3 hours. The resulting precipitate was removed by filtration, washed with propan-2-ol and dried *in vacuo* to give

compound **2.55** as a white solid (28.35 g, 67 %); δ_H (400 MHz, D_2O) 4.20 (dd, 1H, *J* 11, 1.5, lactam C*H*), 4.07 (dd, 1H, *J* 9, 6, pyroglutamic acid C*H*), 3.26-3.12 (m, 2H, lactam C*H*$_2$-NH), 2.46-2.37 (m, 1H, pyroglutamic acid C*H*$_2$-CO), 2.30 (t, 2H, *J* 8, pyroglutamic acid C*H*$_2$-CCOO), 2.01-1.90 (m, 2H, lactam C*H*$_2$-CH, lactam C*H*$_2$-CH_2CH and pyroglutamic acid C*H*$_2$-CO), 1.77 (br.d, 1H, *J* 14, equatorial lactam C*H*$_2$-CH_2NH), 1.71-1.61 (m, 2H, lactam C*H*$_2$-CH_2CH and lactam C*H*$_2$-CH) and 1.31 (br.q, 1H, *J* 12.5, axial lactam C*H*$_2$-CH_2NH); δ_C (100 MHz, D_2O) 181.7, 180.2, 173.8 (*C*=O), 58.3 (pyroglutamic acid *C*H), 52.9 (lactam *C*H), 41.2 (lactam *C*H_2-NH), 29.9 (pyroglutamic acid *C*H_2-CO), 28.2, 27.4, 26.9 (lactam *C*H_2-CH, *C*H_2-CH_2CH, *C*H_2-CH_2NH) and 25.3 (*C*H_2-COO pyroglutamic acid).

2.56, (*R*)-3-Amino-azepan-2-one hydropyroglutamate

Amine **2.52** (38.7 g, 302 mmol) was dissolved in dimethoxyethane (500 mL) and heated to 70 °C. (*R*)-(+)-2-Pyrrolidone-5-carboxylic acid (39.0 g, 302 mmol) was added slowly and the reaction was heated for 3 hours. The resulting precipitate was removed by filtration, washed with propan-2-ol and dried *in vacuo* to give compound **2.56** as a white solid (71.38 g, 91 %); δ_H (400 MHz, D_2O) 4.18 (dd, 1H, *J* 7.5, 2, lactam C*H*), 4.07 (dd, 1H, *J* 10, 6, pyroglutamic acid C*H*), 3.25-3.01 (m, 2H, lactam C*H*$_2$-NH), 2.45-2.35 (m, 1H, pyroglutamic acid C*H*$_2$-CO), 2.30 (t, 2H, *J* 8.5, pyroglutamic acid C*H*$_2$-CCOO), 2.00-1.89 (m, 2H, lactam C*H*$_2$-CH, lactam C*H*$_2$-CH_2CH and pyroglutamic acid C*H*$_2$-CO), 1.80 (br.d, 1H, *J* 13.5, equatorial lactam C*H*$_2$-CH_2NH), 1.70-1.57 (m, 2H, lactam C*H*$_2$-CH_2CH and lactam C*H*$_2$-CH) and 1.31 (br.q, 1H, *J* 12.5, axial lactam C*H*$_2$-CH_2NH); δ_C (100 MHz, D_2O) 181.7, 180.2, 173.8 (*C*=O), 58.3 (pyroglutamic acid *C*H), 52.9 (lactam *C*H), 41.2 (lactam *C*H_2-

NH), 29.7 (pyroglutamic acid $\underline{C}H_2$-CO), 28.2, 27.4, 26.9 (lactam $\underline{C}H_2$-CH, $\underline{C}H_2$-CH_2CH, $\underline{C}H_2$-CH_2NH) and 25.3 ($\underline{C}H_2$-COO pyroglutamic acid).

2.59, (*S*)-3,3,3-Trifluoro-2-methoxy-*N*-((*S*)-2-oxoazepan-3-yl)-2-phenylpropanamide

(*S*)-(-)-α-Methoxy-α-(trifluoromethyl)phenylacetic acid (0.05 g, 0.21 mmol) was dissolved in dichloromethane (1 mL), oxalyl chloride (27 µL, 0.32 mmol) and DMF (1 drop, catalytic) were added and the reaction stirred for 2 hours. The organic solvents were removed *in vacuo* and the product compound **2.57** was dissolved in THF (4 mL) and cooled to 0°C. Pyroglutamic acid salt **2.55** (0.26 g, 0.98 mmol) in H_2O (4 mL) and K_2CO_3 (0.44 g, 3.2 mmol) were added and the reaction was stirred over night. The THF was removed *in vacuo*, ethyl acetate added and was washed with a pH 2 buffer (3 × 15 mL), dried over Na_2SO_4 and reduced *in vacuo* to give compound **2.59** as a white solid (0.046 g, 63 %); δ_H (400 MHz, $CDCl_3$) 8.00 (d, 1H, *J* 6, N$\underline{H}$-CH), 7.56-7.53 (m, 2H, phenyl C$\underline{H}$), 7.40-7.36 (m, 3H, phenyl C$\underline{H}$), 6.05 (br.t, 1H, *J* 4, N$\underline{H}$-CH_2), 4.59 (ddd, 1H, *J* 11, 6, 1.5, lactam C$\underline{H}$), 3.47, 3.39 (br.q, 3H, *J* 1.5, OC$\underline{H_3}$), 3.34-3.20 (m, 2H, lactam C$\underline{H_2}$-NH), 2.05-1.96 (m, 2H, equatorial lactam C$\underline{H_2}$-CH and equatorial lactam C$\underline{H_2}$-CH_2NH), 1.89-1.75 (m, 2H, lactam C$\underline{H_2}$-CH_2CH), 1.51-1.32 (m, 2H, axial lactam C$\underline{H_2}$-CH and C$\underline{H_2}$-CH_2NH).

2.60, (*R*)-3,3,3-Trifluoro-2-methoxy-*N*-((*S*)-2-oxoazepan-3-yl)-2-phenylpropanamide

(*R*)-(+)-α-Methoxy-α-(trifluoromethyl)phenylacetic acid (0.19 g, 0.82 mmol) was dissolved in dichloromethane (4 mL), oxalyl chloride (0.10 mL, 0.15 mmol) and DMF (1 drop, catalytic) were added and the reaction stirred for 2 hours. The organic

solvents were removed *in vacuo* and the product compound **2.58** was dissolved in THF (15 mL) and cooled to 0°C. Pyroglutamic acid salt **2.55** (1.01 g, 3.93 mmol) in H_2O (15 mL) and K_2CO_3 (1.67 g, 12.1 mmol) were added and the reaction was stirred over night. The THF was removed *in vacuo*, ethyl acetate added and was washed with a pH 2 buffer (3 × 25 mL), dried over Na_2SO_4 and reduced *in vacuo* to give compound **2.60** as a colourless oil (0.16 g, 58 %); δ_H (400 MHz, $CDCl_3$) 8.10 (d, 1H, *J* 6, N*H*-CH), 7.49-7.45 (m, 2H, phenyl C*H*), 7.32-7.29 (m, 3H, phenyl C*H*), 7.07 (br.t, 1H, *J* 6, N*H*-CH_2), 4.47 (br.dd, 1H, *J* 11, 7.5, lactam C*H*), 3.46, 3.32 (br.q, 3H, *J* 1.5, OC*H*$_3$), 3.18-3.12 (m, 2H, lactam C*H*$_2$-NH), 2.05 (br.d, 1H, *J* 12.5, equatorial lactam C*H*$_2$-CH), 1.97-1.90 (m, 1H, equatorial lactam C*H*$_2$-CH_2NH), 1.79-1.67 (m, 2H, lactam C*H*$_2$-CH_2CH), 1.47 (q, 1H, *J* 12.5, axial lactam C*H*$_2$-CH) and 1.34-1.22 (m, 1H, C*H*$_2$-CH_2NH).

2.61, (*S*)-Benzyl -2-oxopiperidin-3-ylcarbamate

Amine **2.53** (5.71 g, 50.0 mmol) was dissolved in H_2O (30 mL) and cooled to 0 °C. Benzyl chloroformate (11 mL, 75 mmol) was added. The pH was with Na_2CO_3 until pH 10 was reached and THF (30 mL) was added, the reaction stirred overnight. The THF was removed *in vacuo* and the aqueous layer was extracted with ethyl acetate (3 × 25 mL), diethyl ether (3 × 25 mL), washed with NaCl (3 × 15 mL) and dried over Na_2SO_3. The product was purified by recrystallisation from ethyl acetate and petroleum ether to give compound **2.61** as a white solid (0.91 g, 68 % over 2 steps); The *e.e.* was determined to be > 99 % by chiral HPLC; *mp* 64-65 °C; $[\alpha]^{24}_D$ (c = 1, $CHCl_3$) +35.2; υ_{max}/cm^{-1}: 3323 (N-H), 2950 (saturated C-H), 1749 (C=O ester), 1684 (C=O amide), 1523 (aromatic) and 1259 (C-O); δ_H (400 MHz, $CDCl_3$) 7.37-7.28 (m, 5H, phenyl), 6.85 (br.s, 1H, N*H*-CH_2), 6.03 (d, 1H, *J* 6, CH-N*H*), 5.11 (s, 2H, C*H*$_2$-

phenyl), 4.38-31 (m, 1H, lactam C*H*), 3.22-3.12 (m, 2H, lactam C*H*$_2$-NH), 2.42-2.35 (m, 1H, C*H*$_2$CH), 1.89-1.78 (m, 1H, lactam C*H*$_2$CH) and 1.73-1.53 (m, 2H, lactam C*H*$_2$CH$_2$NH); δ_C (100 MHz, $CDCl_3$) 171.7 (*C*=O amide), 155.4 (*C*=O carbamate), 136.5 (phenyl *C*), 128.5, 128.1 (phenyl *C*H), 67.1 (*C*H$_2$-phenyl), 51.1 (lactam *C*H), 41.7 (lactam *C*H$_2$-NH), 27.0 (lactam *C*H$_2$-CH) and 21.1 (lactam *C*H$_2$-CH$_2$NH). HR ESI ($C_{13}H_{16}N_2O_3Na$ requires 271.1052) found 271.1063.

2.62, (*R*)-Benzyl -2-oxopiperidin-3-ylcarbamate

Amine **2.54** (5.70 g, 50.0 mmol) was dissolved in H_2O (30 mL) and cooled to 0 °C. Benzyl chloroformate (11 mL, 75 mmol) was added. The pH was with Na_2CO_3 until pH 10 was reached and THF (30 mL) was added, the reaction stirred overnight. The THF was removed *in vacuo* and the aqueous layer was extracted with ethyl acetate (3 × 25 mL), diethyl ether (3 × 25 mL), washed with NaCl (3 × 15 mL) and dried over Na_2SO_3. The product was purified by recrystallisation from ethyl acetate and petroleum ether to give compound **2.62** as a white solid (0.91 g, 88 % over 2 steps); The *e.e.* was determined to be > 99 % by chiral HPLC; δ_H (400 MHz, $CDCl_3$) 7.37-7.25 (m, 5H, phenyl), 6.77 (br.s, 1H, N*H*-CH$_2$), 5.91 (d, 1H, *J* 6, N*H*-CH), 5.09 (s, 2H, C*H*$_2$-phenyl), 4.05 (dt, 1H, *J* 10.5, 6.5, C*H*-CO), 3.25-3.19 (m, 2H, lactam C*H*$_2$-NH), 2.45-2.37 (m, 1H, equatorial lactam C*H*$_2$-CH), 1.89-1.78 (m, 2H, lactam C*H*$_2$-CH$_2$NH) and 1.59 (qd, 1H, *J* 12, 5, axial lactam C*H*$_2$-CH); δ_C (100 MHz, $CDCl_3$) 171.7 (lactam C=O), 156.2 (carbamate C=O), 141.2 (phenyl *C*), 128.6, 127.5 (phenyl *C*H), 66.8 (*C*H$_2$-phenyl), 51.6 (lactam *C*H), 41.7 (lactam *C*H$_2$-NH), 27.6 (lactam *C*H$_2$-CH) and 21.0 (lactam *C*H$_2$-CH$_2$NH); ESI *m/z* 80 %, 271.1 (MNa^+) and 80 %, 518.8 (M_2Na^+).

2.63, (*S*)-Benzyl- 2-oxoazepan-3-ylcarbamate

Pyroglutamic acid salt **2.55** (5.25 g, 20.4 mmol) was dissolved in H_2O (20 mL) and cooled to 0 °C. Benzylchloromate (1.4 mL, 10 mmol) was added. The pH was adjusted to pH 10 by the addition of Na_2CO_3 and THF was added, the reaction was stirred for 15 hours. The THF was removed and the aqueous layer extracted with ethyl acetate, washed with $NaHCO_3$, dried over Na_2SO_4 and reduced *in vacuo* to give compound **2.63** as a white solid (2.01 g, 75 %). The *e.e.* was determined to be > 99 % by chiral HPLC; *mp* 121-122 °C; $[\alpha]^{28}_D$ (c = 0.652, MeOH) -0.71; υ_{max}/cm^{-1}: 3239 (N-H), 2914 (saturated C-H), 1723 (C=O carbamate), 1663 (C=O amide) and 1254 (C-O) ester; δ_H (400 MHz, $CDCl_3$) 7.37-7.27 (m, 5H, phenyl C*H*), 6.73 (s, 1H, N*H*-CH_2), 6.21 (d, 1H, *J* 5, N*H*-COO), 5.12 (d, 1H, *J* 12.5, C*H*$_2$-phenyl), 5.08 (d, 1H, *J* 12, C*H*$_2$-benzyl), 4.33 (dd, 1H, *J* 11, 5, C*H*-CO), 3.25-3.17 (m, 2H, lactam C*H*$_2$-NH), 2.09 (br.d, 1H, *J* 13, C*H*$_2$-CH_2CH), 2.03-1.95 (m, 1H, lactam equatorial C*H*$_2$-CH), 1.86-1.70 (m, 2H, lactam C*H*$_2$-CH_2NH and C*H*$_2$-CH_2CH), 1.52 (qd, 1H, *J* 12.5, 3, lactam axial C*H*$_2$-CH) and 1.43-1.30 (m, 1H, C*H*$_2$-CH_2NH); δ_C (100 MHz, $CDCl_3$) 175.6 (*C*=O amide), 155.6 (*C*=O carbamate), 136.6 (phenyl *C*), 128.5, 128.1, 128.0 (phenyl *C*H), 66.7 (*C*H_2-phenyl), 53.7 (*C*H), 42.1 (*C*H_2-NH), 32.0 (*C*H_2-CH), 28.9, 28.0 (*C*H_2-CH_2NH and C*H*$_2$-CH_2CH); ESI *m/*z 100 %, 285.1 (MNa^+). Data is consistent with previously reported data for this compound.[236]

2.64, Cbz (*R*)-3-Amino-azepan-2-one

Pyroglutamic acid salt **2.56** (0.36 g, 1.4 mmol) was dissolved in H_2O (10 mL) and cooled to 0 °C. Benzylchloromate (0.2 mL, 1 mmol) was added. The pH was adjusted to pH 10 by the addition of Na_2CO_3 and THF was added, the reaction was stirred for 15 hours. The reaction was separated and the aqueous layer was extracted

with ethyl acetate and all the organic layers combined. The organic layers were then washed with $NaHCO_3$, dried over Na_2SO_4 and reduced *in vacuo* to give compound **2.64** as a white solid (0.22 g, 79 %); The *e.e.* was determined to be > 99 % by chiral HPLC; *mp* 120-121 °C; $[\alpha]^{31}_D$ (c = 0.63, MeOH) +0.97; υ_{max}/cm^{-1}: 3245 (N-H), 2929 (saturated C-H), 1729 (C=O carbamate), 1664 (C=O amide) and 1249 (C-O) ester; δ_H (400 MHz, $CDCl_3$) 7.36-7.27 (m, 5H, phenyl C*H*), 6.29 (br.t, 1H, N*H*-CH_2), 6.16 (d, 1H, *J* 4, N*H*-CH), 5.11 (d, 1H, *J* 12, C*H*$_2$-phenyl), 5.07 (d, 1H, *J* 12, C*H*$_2$-benzyl), 4.33 (ddd, 1H, *J* 11.5, 6, 1.5, lactam C*H*), 3.30-3.17 (m, 2H, lactam C*H*$_2$-NH), 2.10 (br.d, 1H, *J* 14, equatorial lactam C*H*$_2$-CH), 2.00 (br.d, 1H, equatorial lactam C*H*$_2$-CH_2NH), 1.86-1.70 (m, 2H, lactam C*H*$_2$-CH_2CH), 1.52 (br.q, 1H, *J* 11.5, axial lactam C*H*$_2$-CH) and 1.43-1.32 (m, 1H, C*H*$_2$-CH_2NH); δ_C (100 MHz, $CDCl_3$) 175.5 (*C*=O amide), 155.6 (*C*=O carbamate), 136.6 (phenyl *C*), 128.5, 128.1, 128.0 (phenyl *C*H), 66.7 (*C*H_2-phenyl), 53.7 (*C*H), 42.2 (lactam *C*H_2-NH), 32.1 (lactam *C*H_2-CH), 28.9, 28.0 (lactam *C*H_2-CH_2NH and lactam C*H*$_2$-CH_2CH); ESI *m/*z 100 %, 285.1 (MNa^+) and 34 %, 546.7 (M_2Na^+).

2.65, (*S*)-3-Amino-azepan-2-one hydrogen chloride

Acetyl chloride (37.0 mL, 520 mmol) was dissolved in ethanol (700 mL) and to it pyroglutamic acid salt **2.55** (121.5 g, 472.9 mmol) was added. The reaction was stirred for 2 hours and then filtered to give the HCl salt **2.65** as a white solid (72.14 g, 93 %).

2.66, (*R*)-3-Amino-azepan-2-one hydrogen chloride

Acetyl chloride (24.0 mL, 334 mmol) was dissolved in ethanol (500 mL) and to it pyroglutamic acid salt **2.56** (78.0 g, 303 mmol) was added. The reaction was stirred for 2 hours and then filtered to give the HCl salt **2.66** as a white solid (49.2 g, 99 %).

2.67, Benzyl 4-carboxy benzoate - alternative method of synthesis

Terephthalic acid (15 g, 90 mmol) was dissolved in aqueous KOH (12.0 g, 214 mmol) in 150 ml H_2O. The pH was adjusted to pH 9 by adding HCl drop wise. Ethanol (100 mL) and benzyl chloride (10.5 mL, 100 mmol) were added and the reaction was heated under reflux conditions for 2 hours. After cooling $NaHCO_3$ (10.0 g, 119 mmol) was added and the oily layer was removed by filtration. The filtrate was acidified with concentrated HCl and shaken with ethyl acetate (500 mL). The precipitated terephthalic acid was removed by filtration. The filtrate was separated and the organic layer was washed with NaCl (aq) (3 × 150 mL), dried over Na_2CO_3 and reduced *in vacuo*, the product was then purified by re-crystallisation with hot ethyl acetate and petroleum ether to give compound **2.67** (2.76 g, 10 %); *mp* 175-177 ^oC (decomposed); υ_{max}/cm^{-1}; 2983 (O-H), 1707 (C=O), 1683 (aromatic) and 1259 (ester C-O); δ_H (400 MHz, $CDCl_3$) 8.16 (s, 4H, C*H*-COOH and C*H*-COO), 7.49-7.32 (m, 5H, phenyl) and 5.39 (s, 2H, CH_2-phenyl); δ_C (100 MHz, $CDCl_3$) 176.2 (*C*OOH), 174.9 (*C*OO-benzyl), 138.6 (*C* aryl), 135.8 (*C*-CH_2O), 133.0 (*C* aryl), 129.9, 129.0, 128.3 (phenyl *C*H), 128.1 (*C*H-COOH), 127.1 (*C*H-COO) and 67.9 (*C*H_2-phenyl); HR ESI *m/*z ($C_{15}H_{12}O_4$ requires 255.0663) found 255.0659. Data is consistent with previously reported data for this compound.[237]

2.68, 4-Carboxybenzoate benzaldehyde

4-Carboxybenzaldehyde (0.76 g, 5.1 mmol) was dissolved in DMF (12 mL), this was added to a solution of NaH (0.21 g, 5.3 mmol) in DMF (10 mL) and stirred under nitrogen for 45 minutes. Benzyl bromide (0.7 mL, 5 mmol) was added and the reaction stirred overnight. On completion H_2O was added to the reaction and HCl until pH2 was reached. The product was extracted with ethyl acetate (3 × 15 mL),

the organic layer washed with NaCl and $NaCO_3H$ (3 × 15 mL) then reduced *in vacuo*. The product was stirred in DABCO (15 mL) for 48 hours, pH 2 buffer was added and the product was extracted with ethyl acetate (3 × 15 mL), dried over Na_2CO_3 and reduced *in vacuo* to give compound **2.68** as a yellow oil (0.98 g, 80 %); δ_H (400 MHz, $CDCl_3$) 10.05 (s, 1H, aldehyde *H*), 8.19 (d, 2H, *J* 8, C*H*-CCHO), 7.89 (d, 2H, *J* 8, C*H*-CCOO), 7.48-7.25 (m, 5H, phenyl) and 5.39 (s, 2H, CH_2-phenyl). Data is consistent with previously reported data for this compound.[238]

2.69, Benzyl 4-carboxy benzoate

Sodium chlorite (0.34 g, 3.0 mmol) was dissolved in H_2O (10 mL), this was added dropwise to a solution of aldehyde **2.68** (0.7 g, 3 mmol) and sulfamic acid (0.25 g, 2.6 mmol) in acetonitrile (50 mL) and H_2O (50 mL), the reaction was stirred for one hour. The reaction was quenched with pH 2 buffer (50 mL), extracted with ethyl acetate (3 × 25 mL), washed with NaCl (3 × 15 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by recrystallisation from hot ethyl acetate and petroleum ether to give compound **2.69** as a white solid (0.17 g, 22 %). See alternative method of synthesis for characterisation data.

2.70, 4-Benzyloxycarbonyl benzoyl chloride

Acid **2.69** (2.56 g, 9.99 mmol) was dissolved in dichloromethane (10 mL). Oxalyl chloride (1.3 mL, 15 mmol) and DMF (1 drop, catalytic) was added and the reaction was stirred at room temperature for 24 hours. The oxalyl chloride was removed *in vacuo*. The product compound **2.70** was not isolated and reacted on.

2.71, (*S*)-3-(4'-Benzyloxycarbonyl benzoylamino)-3-amino-azepan-2-one

Acid chloride **2.70** (9.99 mmol) in dichloromethane was cooled on ice and added to pyroglutamic acid salt **2.55** (2.57, 10.0 mmol) in H_2O, the reaction was stirred for 12 hours. The organic layer was separated, the aqueous layer extracted with ethyl acetate (3 × 10 mL) and all the organic layers were combined. The organic layers were washed with pH 2 buffer (3 × 10 mL), dried over Na_2CO_3 and reduced *in vacuo,* the product was purified by re-crystallisation with hot ethyl acetate to give compound **2.71** (0.37 g, 10%); *mp* 160 ºC (decomposed); $[\alpha]^{25}_D$ (c = 0.58, MeOH) +26.03; υ_{max}/cm^{-1}: 2937 (saturated C-H), 1718 (ester C=O), 1666 (amide C=O), 1533 (aromatic) and 1270 (ester C-O); δ_H (400 MHz, $CDCl_3$) 8.11 (d, 2H, *J* 8.5, C*H*-COO), 7.88 (d, 2H, *J* 8.5, C*H*-CONH), 7.77 (d, 1H, *J* 10, N*H*-CH), 7.39-7.54 (m, 5H, phenyl), 6.62 (t, 1H, *J* 5, N*H*-CH_2), 5.36 (s, 2H, C*H*$_2$-phenyl), 4.69 (q, 1H, *J* 5, lactam C*H*), 3.24-3.48 (m, 2H, lactam C*H*$_2$-NH), 2.22 (d, 1H, *J* 13, equatorial lactam C*H*$_2$-CH), 2.00-2.19 (m, 1H, equatorial lactam C*H*$_2$-CH_2NH), 1.79-1.95 (m, 2H, lactam C*H*$_2$-CH_2CH), 1.55 (q, *J* 12.5, axial lactam C*H*$_2$-CH) and 1.41 (q, 1H, *J* 13, axial lactam C*H*$_2$-CH_2NH); δ_C (100 MHz, $CDCl_3$) 175.6 (*C*=O ester), 165.7, 165.33 (*C*=O amide), 138.2, 132.8 (*C* aryl), 135.8 (phenyl *C*), 129.9 (*C*H-CCOO), 128.7, 128.4, 128.2 (phenyl *C*H), 127.2 (*C*H-CCONH), 67.1 (*C*H_2-phenyl), 52.8 (lactam *C*H), 42.2 (lactam *C*H_2-NH), 31.5 (lactam *C*H_2-CH), 28.9 (lactam *C*H_2-CH_2CH) and 28.0 (*C*H_2-CH_2NH); HR ESI *m/*z ($C_{21}H_{22}N_2O_4$ requires 367.1652) found 367.1666.

2.72, (*S*)-3-(4'-Carboxy benzoylamino)- 3-amino-azepan-2-one

Ester **2.71** (0.37 g, 1.0 mmol) was dissolved in THF (20 mL). Palladium activated charcoal (0.04 g, catalytic) was added and the reaction was stirred under hydrogen for 24 hours. The reaction was filtered and the THF was reduced *in vacuo*, the product was purified by re-crystallisation in THF and petroleum ether to give

compound **2.72** as a white solid (0.26 g, 95 %); *mp* 253-257°C (decomposed); $[\alpha]^{26}_{D}$ (c = 0.62, MeOH) +19.64.υ_{max}/cm^{-1}: 2924 (O-H), 1688 (acid C=O) and 1627 (aromatic); δ_H (400 MHz, *d*$_6$-DMSO) 13.19 (s, 1H, O*H*), 8.41 (d, 1H, *J* 7, N*H*-CH), 8.02 (d, 2H, *J* 8, C*H*-CCOOH), 7.95 (d, 2H, *J* 8, C*H*-CCONH), 7.86 (t, 1H, *J* 6, N*H*-CH_2), 4.63 (dd, 1H, *J* 11, 8, lactam C*H*), 3.24 (td, 1H, *J* 8.5, 5 axial lactam C*H*$_2$-NH) 3.10 (m, 1H, equatorial lactam C*H*$_2$-NH), 1.91 (t, 2H, *J* 11, equatorial lactam C*H*$_2$-CH and equatorial lactam C*H*$_2$-CH_2NH), 1.75 (m, 2H, lactam C*H*$_2$-CH_2CH), 1.57 (q, 1H, *J* 11, axial lactam C*H*$_2$-CH) and 1.25 (q, 1H, *J* 12, axial lactam C*H*$_2$-CH_2NH); δ_C (100 MHz, *d*$_6$-DMSO) 174.1 (*C*=O acid), 166.8, 164.5 (*C*=O), 138.0, 133.1 (*C* aryl), 129.3 (*C*H-CCOOH), 127.4 (*C*H-CCONH),), 52.0 (lactam *C*H), 40.6 (lactam *C*H_2-NH), 30.6 (lactam *C*H_2-CH), 28.9 (lactam *C*H_2-CH_2CH) and 27.7 (lactam *C*H_2-CH_2NH); ESI *m/*z 100 %, 299.1 (MNa^+).

2.73, (*S*)- 4'-Benzyloxycarbonyl benzoylamino)-3-amino-tetrahydropyridin-2-one

Acid chloride **2.70** (15 mmol) in dichloromethane was cooled on ice and added to amine **2.53** (15 mmol) in H_2O, the reaction was stirred for 12 hours. The organic layer was separated, the aqueous layer washed with ethyl acetate (3 × 10 mL), the organic layers were washed with pH 2 buffer (3 × 10 mL), dried over Na_2CO_3 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate:MeOH 75:25:0 to 0:90:10) to give compound **2.73** (0.93 g, 18 %); *mp* 139-140 °C;$[\alpha]^{24}_{D}$ (c = 0.27, $CHCl_3$) +60.09; υ_{max}/cm^{-1}: 3240 (N-H), 2945 (saturated C-H), 1724 (ester C=O), 1673 (amide C=O), 1548 (aromatic) and 1278 (ester C-O); δ_H (400 MHz, $CDCl_3$) 8.03 (d, 2H, *J* 7.5, C*H*-CCOO), 7.83 (d, 2H, *J* 7.5, C*H*-CCONH), 7.70 (d, 1H, *J* 6, N*H*-CH), 7.24-7.29 (m, 5H, phenyl), 6.85 (br.s, 1H, N*H*-CH_2), 5.33 (s, 2H, C*H*$_2$-phenyl), 4.39 (dt, 1H, *J* 11.5, 5.5, lactam C*H*),

3.31-3.26 (m, 2H, lactam C*H*$_2$-NH), 2.50 (ddt, 1H, *J* 4.5, 5, 13, lactam C*H*$_2$-CH), 1.2-1.84 (m, 2H, lactam C*H*$_2$-CH$_2$CH) and 1.73-1.62 (m, 1H, lactam C*H*$_2$-CH), δ_C (100 MHz, $CDCl_3$) 172.0 (*C*=O ester), 166.6, 165.7 (*C*=O amide), 138.0 (*C* phenyl), 135.7, 132.7 (*C* aryl), 129.8 (*C*H-CCOO), 128.7, 128.4, 128.3 (phenyl *C*H), 127.3 (*C*H-CCONH), 67.1 (*C*H$_2$-phenyl), 50.9 (lactam *C*H), 41.7 (lactam *C*H$_2$-NH), 27.1 (lactam *C*H$_2$-CH) and 21.2 (lactam *C*H$_2$-CH$_2$NH); ESI *m/*z 100 %, 375.1 (MNa^+) and 29 %, 353.1 (MH^+); HR ESI *m/*z ($C_{20}H_{20}N_2O_4Na^+$ requires 375.1315) found 375.1313.

2.74, (*S*)-Benzyl 1-(4-(2-oxoazepan-3-ylcarbamoyl)benzoyl)piperidine-4-carboxylate

Acid **2.72** (0.13 g, 0.45 mmol) was dissolved in dichloromethane (5 mL) and cooled to 0 ºC. HATU (0.17 g, 0.45 mmol) was added and the reaction stirred for 10 minutes. Amine **2.77** (0.5 mmol) and triethylamine (0.30 mL, 1.4 mmol) were added and the reaction stirred for 15 hours. The dichloromethane was removed *in vacuo*, the product dissolved in ethyl acetate (20 mL), washed with pH 2 buffer (3 × 15 mL) and ½ saturated sodium bicarbonate (3 × 15 mL), dried over Na_2CO_3 and reduced *in vacuo*. The product was purified silica column chromatography petroleum ether:ethyl acetate:MeOH (60:40:0 to 0:90:10) to give compound **2.74** as a colourless oil (0.10 g, 47 %); δ_H (400 MHz, $CDCl_3$) 7.85 (d, 2H, *J* 7.5, aryl C*H*), 7.69 (d, 1H, *J* 5.5, N*H*-CH), 7.42 (d, 2H, *J* 8, aryl C*H*), 7.37-7.27 (m, 5H, phenyl), 6.80 (br.t, 1H, *J* 5, N*H*-CH$_2$), 5.11 (s, 2H, C*H*$_2$-phenyl), 4.68 (q, 1H, *J* 5, lactam C*H*), 4.55-4.40 (m, 1H, equatorial C*H*$_2$-N), 3.70-3.52 (m, 1H, equatorial C*H*$_2$-N), 3.34-3.19 (m, 2H, lactam C*H*$_2$-NH), 3.12-2.93 (m, 2H, axial C*H*$_2$-N), 2.61 (tt, 1H, *J* 10, 4, C*H*-CH$_2$CH$_2$N), 2.19 (d, 1H, *J* 14, equatorial lactam C*H*$_2$-CH), 2.11-1.96 (m, 2H,

lactam C<u>*H*</u>$_2$-CH_2CH), 1.94-1.60 (m, 5H, equatorial lactam C<u>*H*</u>$_2$-CH_2NH and CH_2-CH_2N), 1.53 (q, 1H, *J* 12, axial lactam C<u>*H*</u>$_2$-CH) and 1.39 (q, 1H, *J* 12, axial lactam C<u>*H*</u>$_2$-CH_2NH); δ_C (100 MHz, $CDCl_3$) 175.7 (C=O ester), 173.8, 169.5, 165.4 (C=O), 138.9 (<u>*C*</u>H-CON), 135.8 (<u>*C*</u>-CH_2O), 135.3 (<u>*C*</u>-CONH), 128.6, 128.4, 128.1 (phenyl <u>*C*</u>H), 127.4, 127.0 (aryl <u>*C*</u>H), 66.5 (<u>*C*</u>H_2-phenyl), 52.7 (lactam <u>*C*</u>H), 46.9 (<u>*C*</u>H_2-NH), 42.1 (lactam <u>*C*</u>H_2-N), 41.5 (<u>*C*</u>H_2-N), 40.9 (<u>*C*</u>H-COO-benzyl), 31.5 (lactam <u>*C*</u>H_2-CH), 28.9 (lactam <u>*C*</u>H_2-CH_2CH), 28.6 (<u>*C*</u>H_2-CH_2N) and 28.0 (lactam <u>*C*</u>H_2-CH_2NH).

2.75, 1-(*tert*-Butoxycarbonyl)piperidine-4-carboxylic acid

Isonipecotic acid (15.5 g, 120 mmol) was dissolved in H_2O with Na_2CO_3 (21.16 g, 199.6 mmol) and dioxane (50 mL). Di-*tert*-butyl-dicarbonate (26.0 g, 119 mmol) was added and the reaction was stirred for 24 hours. The organic solvents were reduced *in vacuo* and the reaction acidified to pH 2 with HCl. Diethyl ether (80 mL) was added and the resulting precipitate was removed through filtration. The aqueous layer was extracted with ethyl acetate (3 × 25 mL), the organic layers were then combined and dried over $NaSO_4$ and reduced *in vacuo* to give compound **2.75** as a white solid (19.11 g, 69 %); *mp* 145-147 ºC; υ_{max}/cm^{-1}: 3192 (O-H), 2973 (saturated C-H), 1732 (carbamate C=O), 1655 (amide C=O) and 1154 (ester C-O); δ_H (500 MHz, $CDCl_3$) 9.65 (OH), 3.99 (br.s, 2H, equatorial C<u>*H*</u>$_2$-N), 2.84 (t, 2H, *J* 11.5, axial C<u>*H*</u>$_2$-N), 2.46 (tt, 1H, *J* 11, 4, C<u>*H*</u>-COOH), 1.88 (2H, d, *J* 12.5, equatorial C<u>*H*</u>$_2$-CH_2N), 1.62 (qd, 2H, *J* 10.5, 4.5, axial C<u>*H*</u>$_2$-CH_2N) and 1.43 (s, 9H, C(<u>*C*</u>H_3)$_3$); δ_C (125 MHz, $CDCl_3$) 180.1 (<u>*C*</u>=O acid), 154.8 (<u>*C*</u>=O carbamate), 79.9 (<u>*C*</u>$(CH_3)_3$, 43.3 (<u>*C*</u>H_2-N), 40.8 (<u>*C*</u>H-COOH), 28.7 (C(<u>*C*</u>H_3)$_3$ and 28.2 (<u>*C*</u>H_2-CH_2N). Data is consistent with previously reported data for this compound.[239]

2.76, 4-Benzyl-1-*tert*-butyl piperidine-1,4-dicarboxylate

Acid **2.75** (18.0 g, 78.5 mmol) was dissolved in DMSO (50 mL) and to this KOH (4.84 g, 86.4 mmol) was H_2O (10 mL) were added. Benzyl bromide (9.30 mL, 78.5 mmol) was added and the reaction stirred for 15 hours. H_2O (100 mL) was added and the product was extracted with ethyl acetate (3 × 50 mL), washed with ½ saturated sodium bicarbonate (3 × 50 mL) to give compound **2.76** as a colourless oil (16.6 g, 66 %); υ_{max}/cm^{-1}: 2975 (saturated C-H), 1732 (ester C=O), 1688 (carbamate C=O) and 1154 (ester C-O); δ_H (400 MHz, $CDCl_3$) 7.38-7.28 (m, 5H, phenyl), 5.11 (s, 2H, C$\underline{H}_2$-phenyl), 4.01 (br.d, 2H, *J* 10, equatorial C$\underline{H}_2$-N), 2.81 (br.t, 2H, *J* 12, axial C$\underline{H}_2$-N), 2.49 (tt, 1H, *J* 11, 4, C$\underline{H}$-CO), 1.89 (dd, 2H, *J* 13, 3, equatorial C$\underline{H}_2$-CH_2-N), 1.64 (dtd, 2H, *J* 14, 12, 5, axial C$\underline{H}_2$-CH_2N) and 1.46 (s, 9H, C(C$\underline{H}_3$)$_3$); δ_C (100 MHz, $CDCl_3$) 174.3 (ester C=O), 154.7 (carbamate C=O), 136.0 (phenyl $\underline{C}$), 128.6, 128.3, 128.1 (phenyl $\underline{C}$H), 79.6 ($\underline{C}(CH_3)_3$), 66.3 ($\underline{C}H_2$-phenyl), 42.8 ($\underline{C}H_2$-N), 41.2 ($\underline{C}$H-CO), 28.4 (C($\underline{C}H_3$)$_3$) and 28.0 ($\underline{C}H_2$-CH_2-N); ESI *mz* 100 %, 342.2 (MNa^+). Data is consistent with previously reported data for this compound.[240]

2.77, 4-Benzyl-piperidine-4-carboxylate hydrogen chloride

Carbamate **2.76** (50 mmol) was dissolved in 4M HCl in dioxane (10 mL) and stirred for 4 hours. The organic solvents were removed *in vacuo* to give compound, **2.77** as a white solid (12.7 g, 99 %); *mp* 146-147 °C; υ_{max}/cm^{-1}: 2938 (saturated C-H), 1737 (ester C=O), 1596 (aromatic) and 1199 (ester C-O); δ_H (d^6-DMSO) 9.60 (br.s, 1H, N$\underline{H}_2$Cl), 9.44 (br.s, 1H, N$\underline{H}_2$Cl), 7.36-7.29 (m, 5H, phenyl), 5.12 (s, 2H, C$\underline{H}_2$-phenyl), 3.35 (br.s, 2H, equatorial C$\underline{H}_2$-N), 3.02 (br.s, 2H, axial C$\underline{H}_2$-N), 2.54 (quintet, 1H, *J* 4.5, C$\underline{H}$-COO-benzyl) and 2.25-2.07 (m, 4H, C$\underline{H}_2$-CH_2-N). Data is consistent with previously reported data for this compound.[241]

2.78, 3-(4-(Benzyloxycarbonyl)piperidin-1-ylsulfonyl)benzoic acid

Amine **2.77** (2.63 g, 10.3 mmol) was dissolved in dichloromethane (40 mL) and triethylamine (5.6 mL, 40 mmol), the reaction was cooled on ice. 3-(Chlorosulfonyl)benzoic acid (2.21 g, 10.0 mmol) was added and the reaction was stirred for 48 hours. Further dichloromethane (40 mL) was and the reaction which was washed with pH 2 buffer (3 × 25 mL), dried over $NaSO_4$ and reduced *in vacuo.* The product was purified by recrystallisation from hot ethyl acetate to give compound **2.78** as a white solid (1.74 g, 43 %); *mp* 166-167 °C; υ_{max}/cm^{-1}: 2926 (O-H), 1729, 1677 (acid C=O), 1336, 1134 (SO_2), 1160 and 1115 (C-O); *Anal*. Calcd for $C_{20}H_{21}NO_6S$: C, 59.54; H, 5.25; N, 3.47. Found: C, 59.62; H, 5.19; N, 3.51; δ_H (400 MHz, d^6-DMSO) 8.28 (d, 2H, *J* 7.5, C*H*-CCOO), 8.22 (s, 1H, OCC-C*H*-SO_2), 8.01 (d, 1H, *J* 8, C*H*-CSO_2), 7.82 (t, 1H, *J* 7.5, C*H*-CH-CSO_2), 7.40-7.30 (m, 5H, phenyl), 5.09 (s, 2H, CH_2-phenyl, 3.55 (br.d, 2H, *J* 11.5, equatorial CH_2-N), 2.55-2.46 (m, 3H, axial CH_2-N and C*H*-CH_2), 1.96 (dd, 2H, *J* 12.5, 3, equatorial CH_2-CH_2N) and 1.62 (qd, 2H, *J* 12, 4, axial CH_2-CH_2N); δ_c (100 MHz, d^6-DMSO) 173.31 (*C*=O acid), 166.0 (*C*=O ester), 136.2, 136.1, 132.0 (*C*-SO_2, *C*-COOH and *C*-CH_2O), 133.6 (*C*H-CCOOH), 131.4 (*C*H-CSO_2), 130.2 (*C*H-CH-CSO_2), 128.4 (OCC-*C*H-SO_2), 128.0, 127.7 (phenyl), 65.5 (CH_2-phenyl), 44.9 (*C*H_2-N), 38.7 (*C*H-COO-benzyl) and 27.0 (*C*H_2-CH_2N); ESI *m/*z 100 %, 426.1 (MNa^+) and 7 %, 404.1 (MH^+).

2.79, 4-(4-(Benzyloxycarbonyl)piperidin-1-ylsulfonyl)benzoic acid

Amine **2.77** (3.99 g, 15.6 mmol) was dissolved in dichloromethane and triethylamine (7.8 mL, 56 mmol). The reaction was cooled on ice, 4-(chlorosulfonyl) benzoic acid (3.10 g, 14.1 mmol) was added and the reaction stirred overnight. The reaction was acidified and the resulting precipitate was removed by filtration, the remaining

organic solvents were removed *in vacuo* to give compound **2.79** as a white solid (5.0 g, 88 %); υ_{max}/cm^{-1}: 2965 (saturated C-H), 1727 (ester C=O), 1687 (amide C=O), 1653 (aromatic), 1336, 1166 (SO_2) and 1280 (C-O); δ_H (400 MHz, d^6-DMSO) 8.17 (d, 2H, *J* 8, C*H*-CCOOH), 7.86 (d, 2H, *J* 8.5, C*H*-CSO_2), 7.39-7.28 (m, 5H, phenyl), 5.07 (s, 2H, C*H*$_2$-phenyl), 3.52 (dt, 2H, *J* 12, 4, equatorial C*H*$_2$-N), 2.55-2.43 (m, 3H, axial C*H*$_2$-N and C*H*-CH_2CH_2N), 1.93 (dd, 2H, *J* 14, 3, equatorial C*H*$_2$-CH_2N) and 1.60 (qd, 2H, *J* 11,4, axial CH_2-CH_2N); δ_c (100 MHz, d^6-DMSO) 173.3 (*C*=O acid), 166.2 (*C*=O ester), 139.3, 136.1, 134.7 (*C*-SO_2, *C*-COOH and phenyl *C*), 130.2 (*C*-COOH or *C*-CSO_2), 128.4 (phenyl *C*H), 128.0 (*C*-COOH or *C*-CSO_2), 127.7, 127.6 (phenyl *C*H), 65.5 (*C*H_2-phenyl), 45.0 (*C*H_2-N), 38.7 (*C*H-CH_2-CH_2N) and 27.0 (*C*H_2-CH_2N); ESI *m/*z 100 %, 426.0 (MNa^+).

2.80, (*S*)-Benzyl 1-(3-(2-oxoazepan-3-ylcarbamoyl)phenylsulfonyl)piperidine-4-carboxylate

Acid **2.78** (3.01 g, 7.46 mmol) was dissolved in dichloromethane and cooled to 0 °C. HATU (2.80 g, 7.36 mmol) was added and the reaction stirred for 1 hour. Lactam **2.65** (2.05 g, 7.97 mmol) in H_2O and triethylamine (3.1 mL, 22 mmol) and the reaction was stirred overnight. Ethyl acetate was added, the organic layer was washed with pH 2 buffer (3 × 25 mL) and ½ saturated sodium bicarbonate, dried over Na_2CO_3 and reduced *in vacuo.* The product was purified by silica column chromatography (petroleum ether:ethyl acetate:MeOH 50:50:0 to 0:70:30 to give compound **2.80** as a white solid (2.88 g, 75 %) *mp* 78-80 º C; υ_{max}/cm^{-1}: 3273 (N-H), 2930 (saturated C-H), 1726 (C=O ester), 1644 (C=O amide), 1333, 1150 (SO_2) and 1287 (C-O); δ_H (400 MHz, $CDCl_3$) 8.21 (s, 1H, OCC-C*H*-SO_2), 8.06 (d, 2H, *J* 8, C*H*-CCO or C*H*-CSO_2), 7.88-7.84 (m, 2H, C*H*-CCO or C*H*-CSO_2 and N*H*-CH), 7.61 (t, 1H, *J* 8, C*H*-CH-CSO_2), 7.37-7.27 (m, 5H, phenyl), 6.70 (t, 1H, *J* 6, N*H*-

CH_2), 5.09 (s, 2H, C<u>*H*</u>$_2$-phenyl), 4.72 (dd, 1H, *J* 11, 6, lactam C<u>*H*</u>-CO), 3.66 (br.d, 2H, *J* 12, equatorial C<u>*H*</u>$_2$-N), 3.35-3.30 (m, 2H, lactam C<u>*H*</u>$_2$-NH), 2.50 (dt, 2H, *J* 11.5, 2.5, axial C<u>*H*</u>$_2$-N), 2.32 (tt, 1H, *J* 10.5, 4, C<u>*H*</u>-CH_2-CH_2N), 2.19 (br.d, 1H, *J* 12.5, equatorial C<u>*H*</u>$_2$-CH), 2.10-1.97 (m, axial C<u>*H*</u>$_2$-CH_2N and equatorial lactam C<u>*H*</u>$_2$-CH_2NH), 1.92-2.77 (m, 4H, axial C<u>*H*</u>$_2$-CH_2N and lactam C<u>*H*</u>$_2$-CH_2CH), 1.59 (q, 1H, *J* 12.5, equatorial lactam C<u>*H*</u>$_2$-CH) and 1.43 (br.q, 1H, *J* 11.5, axial lactam C<u>*H*</u>$_2$-CH_2NH); δ_c (100 MHz, CDCl3) 175.5, 173.5 (<u>*C*</u>=O ester and amide), 164.6 (C=O lactam), 136.9, 135.7, 135.5 (<u>*C*</u>-COO, <u>*C*</u>-SO_2, <u>*C*</u>-CH_2-O), 131.3 (<u>*C*</u>H-CSO_2 and <u>*C*</u>H-COO), 130.2 (<u>*C*</u>H-CSO_2 and <u>*C*</u>H-COO), 129.5 (<u>*C*</u>H-CH-CSO_2), 128.6 (phenyl *m*-<u>*C*</u>H), 128.3 (phenyl *p*-<u>*C*</u>H), 128.0 (phenyl *o*-<u>*C*</u>H), 126.3 (OCC-<u>*C*</u>H-SO_2), 66.5 (C<u>*H*</u>$_2$-phenyl), 52.8 (lactam C<u>*H*</u>), 47.4 (lactam <u>*C*</u>H_2-NH), 45.4 (<u>*C*</u>H_2-N), 39.9 (<u>*C*</u>H-CH_2CH_2N), 31.4 (lactam <u>*C*</u>H_2-CH), 28.8 (lactam <u>*C*</u>H_2-CH_2CH), 28.0 (<u>*C*</u>H_2-CH_2N) and 27.4 (lactam <u>*C*</u>H_2-CH_2NH); ESI *m/*z 100 %, 536.3 (MNa^+).

2.81, (*S*)-Benzyl 1-(4-(2-oxoazepan-3-ylcarbamoyl)phenylsulfonyl)piperidine-4-carboxylate

Acid **2.79** (6.00 g, 14.9 mmol) was dissolved in dichloromethane and cooled to 0 °C. HATU (5.38 g, 14.2 mmol) was added and the reaction stirred for 1 hour. Amine **2.65** (3.00 g, 18.3 mmol) and triethylamine (5.8 mL, 21 mmol) were added and the reaction stirred overnight. The reaction was washed with H_2O, and dried over Na_2CO_3 and reduced *in vacuo* to give a white solid. The product was purified by silica column chromatography (petroleum ether:ethyl acetate:MeOH 50:50:0 to 0:70:30) to compound **2.81** as a white solid (4.35 g, 57 %) *mp* 187-188 ° C; $[\alpha]^{22}_D$ (c = 0.352, chloroform) -37.43; υ_{max}/cm^{-1}: 3300 (N-H), 1725 (C=O ester), 1644 (C=O amide) and 1333, 1161 (SO_2); δ_H (400 MHz, d_6- DMSO) 8.54 (d, 1H, *J* 7, N<u>*H*</u>-CH), 8.10 (d, 2H, *J* 8.5, C<u>*H*</u>-CCO), 7.88 (t, 1H, *J* 6, N<u>*H*</u>-CH_2), 7.84 (d, 2H, *J* 8, C<u>*H*</u>-

CSO_2), 7.38-7.28 (m, 5H, phenyl), 5.07 (s, 2H, C<u>*H*</u>$_2$-phenyl), 4.66 (dd, 1H, *J* 11,7, lactam C<u>*H*</u>), 3.52 (br.d, 2H, *J* 12, equatorial C<u>*H*</u>$_2$-N), 3.26 (td, 1H, *J* 10.5,5, lactam axial C<u>*H*</u>$_2$-NH), 3.16-3.04 (m, 1H, lactam equatorial C<u>*H*</u>$_2$-NH), 2.52-2.42 (m, 3H, axial C<u>*H*</u>$_2$-N and C<u>*H*</u>-CH_2CH_2N), 1.97-1.84 (m, 4H, C<u>*H*</u>$_2$-CH_2N), 1.83-1.53 (m, 5H, lactam C<u>*H*</u>$_2$-CH_2NH, C<u>*H*</u>$_2$-CH_2CH and equatorial C<u>*H*</u>$_2$-CH) and 1.26 (q, 1H, *J* 12, axial lactam C<u>*H*</u>$_2$-CH); δ_C (100 MHz,d^6-DMSO) 174.0, 173.3 (<u>*C*</u>=O ester and amide), 164.0 (<u>*C*</u>=O lactam), 138.2, 137.8, 136.1 (aryl <u>*C*</u>), 128.4 (<u>*C*</u>H-CCO), 128.3 (<u>*C*</u>H-CSO_2), 128.0, 127.7, 127.5 (phenyl <u>*C*</u>H), 66.5 (C<u>*H*</u>$_2$-phenyl), 52.2 (lactam C<u>*H*</u>), 45.0 (lactam <u>*C*</u>H_2-NH), 40.6 (<u>*C*</u>H_2-N), 39.9 (<u>*C*</u>H-CH_2CH_2N), 30.5 (lactam <u>*C*</u>H_2-CH), 28.9 (lactam <u>*C*</u>H_2-CH_2CH), 27.7 (<u>*C*</u>H_2-CH_2N) and 27.1 (lactam <u>*C*</u>H_2-CH_2NH); ESI *m/*z 100 %, 536.2 (MNa^+).

2.82, (*S*)-Benzyl 1-(3-(2-oxopiperidin-3-ylcarbamoyl)phenylsulfonyl)piperidine-4-carboxylate

Lactam **2.53** (1.71 g, 15.0 mmol) and K_2CO_3 (4.31 g, 31.2 mmol) was dissolved in H_2O (25 mL) and cooled to 0 °C. Acid **2.78** (8 mmol) in dichloromethane, oxalyl chloride (1.4 mL, 16 mmol) and DMF (1 drop, catalytic) were added. The reaction was stirred for 2 hours, reduced *in vacuo*, the acid chloride re-dissolved in dichloromethane and slowly added to the lactam solution. The reaction was stirred overnight, the dichloromethane removed *in vacuo*. The reaction was extracted with ethyl acetate and washed with pH 2 buffer (3 × 15 mL) and ½ saturated $NaHCO_3$ (3 × 15 mL), dried over Na_2SO_3 and reduced *in vacuo.* The product was purified by silica column chromatography (petroleum ether:ethyl acetate:MeOH 50:50:0 to 0:70:30) to give compound **2.82** as a white solid (3.76 g, 50 %); δ_H (300 MHz, $CDCl_3$) 8.18 (s, 1H, OCC-C<u>*H*</u>-SO_2), 8.06 (d, 1H, *J* 8, C<u>*H*</u>-CCO or C<u>*H*</u>-CSO_2), 7.96 (d, 1H, *J* 6.5, N<u>*H*</u>-CH), 7.83 (d, 1H, *J* 8, C<u>*H*</u>-CCO or C<u>*H*</u>-CSO_2), 7.57 (t, 1H, *J* 8,

C<u>*H*</u>-CH-CSO_2), 7.36-7.27 (m, 5H, phenyl), 6.52 (br.s, 1H, N<u>*H*</u>-CH_2), 5.09 (s, 2H, C<u>*H*</u>$_2$-phenyl), 4.50 (q, 1H, *J* 6.5, lactam C<u>*H*</u>), 3.65 (br.dd, 2H, *J* 11.5, 3.5, equatorial C<u>*H*</u>$_2$-N), 3.34 (br.td, 2H, *J* 6.5, 2, lactam C<u>*H*</u>$_2$-NH), 2.47 (br.t, 2H, *J* 11.5, axial C<u>*H*</u>$_2$-N), 2.33 (tt, 1H, *J* 12.5, 4, C<u>*H*</u>-CH_2-CH_2N), 2.04-1.90 (m, 4H, C<u>*H*</u>$_2$-CH_2N) and 1.89-1.72 (m, 4H, lactam C<u>*H*</u>$_2$-CH and C<u>*H*</u>$_2$-CH_2NH); *m/*z ($C_{25}H_{29}N_3O_6SNa^+$ requires 522.1675) found 522.2.

2.09, (*S*)-1-(3-(2-Oxoazepan-3-ylcarbamoyl)phenylsulfonyl)piperidine-4-carboxylic acid

Ester **2.80** (1.64, 3.19 mmol) was dissolved in MeOH (20 mL). Palladium activated charcoal (0.2 g, catalytic) was added and the reaction stirred under hydrogen for 48 hours. The reaction was filtered through celite and reduced *in vacuo* to give compound **2.09** as a white solid (0.83 g, 61%); *mp* 170-175 °C (decomposed); υ_{max}/cm^{-1}: 3267 (N-H), 2937 (saturated C-H), 1724 (C=O acid), 1627 (C=O amide), 1539 (aromatic), 1335, 1172 (SO_2) and 1272 (C-O acid); δ_H (400 MHz, $CDCl_3$) 8.16 (s, 1H, OCC-C<u>*H*</u>-SO_2), 8.04 (d, 1H, *J* 8, C<u>*H*</u>-CCO or C<u>*H*</u>-CSO_2), 7.87 (d, 1H, *J* 8, C<u>*H*</u>-CCO or C<u>*H*</u>-CSO_2), 7.81 (d, 1H, *J* 6, N<u>*H*</u>-CH), 7.60 (t, 1H, *J* 8, C<u>*H*</u>-CH-CSO_2), 7.39 (d, 1H, *J* 6, N<u>*H*</u>-CH_2), 4.74 (dd, 1H, *J* 10.5, 6, lactam C<u>*H*</u>), 3.66 (br.d, 2H, *J* 10, equatorial C<u>*H*</u>$_2$-N), 3.31 (br.s, 2H, lactam C<u>*H*</u>$_2$-NH), 2.46 (q, 2H, *J* 9, axial C<u>*H*</u>$_2$-N), 2.23 (br.t, 1H, *J* 10.5, C<u>*H*</u>-CH_2CH_2N), 2.16 (d, 2H, *J* 13, equatorial lactam C<u>*H*</u>$_2$-CH), 2.08-1.71 (m, 7H,lactam C<u>*H*</u>$_2$-CH_2NH, C<u>*H*</u>$_2CH_2$CH and C<u>*H*</u>$_2$-CH_2N), 1.57 (q, 1H, *J* 12, axial lactam C<u>*H*</u>$_2$-CH) and 1.47-1.34 (1H, m, C<u>*H*</u>$_2$-CH_2NH); δ_C (100 MHz, $CDCl_3$) 178.0 (C=O acid), 176.5 (C=O amide), 164.9 (C=O lactam), 137.0, 135.4 (<u>*C*</u>SO_2 and <u>*C*</u>CO), 131.5, 130.4 (<u>*C*</u>H-CCO and <u>*C*</u>H-CSO_2), 129.6 (<u>*C*</u>H-CH-CSO_2), 126.1 (OCC-<u>*C*</u>H-SO_2), 52.7 (lactam <u>*C*</u>H), 45.5 (<u>*C*</u>H_2-N), 42.2 (lactam <u>*C*</u>H_2-NH), 39.8

(<u>C</u>H-CH_2CH_2N), 31.3 (lactam <u>C</u>H_2-CH), 28.5 (lactam <u>C</u>H_2-CH_2CH), 27.9 (<u>C</u>H_2-CH_2N), and 27.4 (lactam <u>C</u>H_2-CH_2NH).

2.83, (*S*)-1-(4-(2-Oxoazepan-3-ylcarbamoyl)phenylsulfonyl)piperidine-4-carboxylic acid

Ester **2.81** (2.05 g, 4.00 mmol) was dissolved in THF (10 mL) and MeOH (10 mL). Palladium activated charcoal (0.2 g, catalytic) was added and the reaction stirred under hydrogen for 48 hours. The reaction was filtered through celite and reduced *in vacuo* to give compound **2.83** as a white solid (1.58 g, 93%); δ_H (400 MHz, d_6-DMSO) 8.53 (d, 1H, *J* 7, N<u>*H*</u>-CH), 8.08 (d, 2H, *J* 8, C<u>*H*</u>-CCO), 7.87-7.81 (m, 3H, C<u>*H*</u>-CSO_2 and N<u>*H*</u>-CH_2), 4.63 (dd, 1H, *J* 10.5, 7, lactam C<u>*H*</u>), 3.62-3.78 (m, 2H, equatorial C<u>*H*</u>$_2$-N), 3.51-3.44 (m, 2H, lactam axial C<u>*H*</u>$_2$-NH), 2.45 (br.t, 2H, *J* 11, C<u>*H*</u>$_2$-N axial), 2.27 (tt, 1H, *J* 10, 2, C<u>*H*</u>-CH_2CH_2N), 1.96-1.83 (m, 4H, C<u>*H*</u>$_2$-CH_2N), 1.81-1.67 (m, 2H, lactam C<u>*H*</u>$_2$-CH_2CH), 1.50-1.04 (m, 3H, C<u>*H*</u>$_2$-CH_2NH and equatorial C<u>*H*</u>$_2$-CH) and 1.25 (q, 1H, *J* 13.5, axial lactam C<u>*H*</u>$_2$-CH); ESI *m/*z ($C_{19}H_{25}N_3O_6SNa^+$ requires 446.14) found 446.1.

2.84, (*S*)-1-(3-(2-Oxopiperidin-3-ylcarbamoyl)phenylsulfonyl)piperidine-4-carboxylic acid

Ester **2.82** (3.76 g, 7.50 mmol) was dissolved in MeOH (50 mL), palladium activated charcoal (0.4 g, catalytic) was added and the reaction was stirred under hydrogen for 48 hours. The reaction was filtered through celite and reduced *in vacuo* to give compound **2.84** as a white solid (2.21 g, 72 %); δ_H (300 MHz, d^6- DMSO) 9.00 (d, 1H, *J* 8, N<u>*H*</u>-CH), 8.20 (s, 1H, OCC-C<u>*H*</u>-SO_2), 8.19 (d, 1H, *J* 8, C<u>*H*</u>-CCO or C<u>*H*</u>-CSO_2), 7.89 (d, 1H, *J* 8, C<u>*H*</u>-CCO or C<u>*H*</u>-CSO_2), 7.76 (t, 1H, *J* 8, C<u>*H*</u>-CH-CSO_2), 7.72 (br.s, 1H, N<u>*H*</u>-CH_2), 4.40 (br.q, 1H, *J* 7.5, lactam C<u>*H*</u>), 3.50 (br.d, 2H, *J* 10.5,

equatorial C<u>*H*</u>$_2$-N), 3.31-3.32 (m, 2H, lactam C<u>*H*</u>$_2$-NH), 2.43 (br.t, 2H, *J* 10.5, axial C<u>*H*</u>$_2$-N), 2.28 (tt, 1H, *J* 10.5, 3.5, C<u>*H*</u>-CH_2CH_2N), 2.04-1.96 (m, 1H, lactam C<u>*H*</u>$_2$-CH_2NH and C<u>*H*</u>$_2$-CH_2N), 1.92-1.77 (m, 5H, lactam C<u>*H*</u>$_2$-CH_2NH and C<u>*H*</u>$_2$-CH_2N) and 1.55 (br. q, 2H, *J* 13, lactam C<u>*H*</u>$_2$-CH_2CH).

2.08, (*S*)-*N*-(2-(1*H*-indol-3-yl)ethyl)-1-(3-(2-oxoazepan-3-ylcarbamoyl) phenylsulfonyl)piperidine-4-carboxamide

Acid **2.09** (0.83 g, 2.0 mmol) was dissolved in dichloromethane (15 mL) and cooled to 0° C. HATU (0.78 g, 2.1 mmol) was added and the mixture was stirred for 4 hours. Tryptamine (0.34 g, 2.1 mmol) and triethylamine (0.90 mL, 6.4 mmol) were added and the reaction was stirred for 48 hours. The dichloromethane was removed *in vacuo* and the product was dissolved in ethyl acetate and was washed with a pH 2 buffer (3 × 15 mL) and ½ saturated $NaHCO_3$ (3 × 15 mL). The organic layer was reduced *in vacuo* and the product was purified by silica column chromatography (petroleum ether:ethyl acetate:MeOH 50:50:0 to 0:70:30) to give compound **2.08** as a white solid (0.23 g, 20 %) *mp* 127-129 °C; $[\alpha]^{24}_D$ (c = 0.117, MeOH) + 10.68; υ_{max}/cm^{-1}: 3384 (N-H), 2929 (saturated C-H), 1639 (C=O amide), 1517 (aromatic) and 1334, 1149 (SO_2); δ_H (300 MHz, $CDCl_3$) 8.63 (br.s, 1H, N<u>*H*</u>-indole), 8.19 (s, 1H, OCC-C<u>*H*</u>-SO_2), 8.03 (d, 1H, *J* 7.5, C<u>*H*</u>-CCO or C<u>*H*</u>-CSO_2), 7.86 (d, 1H, N<u>*H*</u>-CH), 7.83 (d, 1H, *J* 8, C<u>*H*</u>-CCO or C<u>*H*</u>-CSO_2), 7.57 (t, 1H, *J* 8, C<u>*H*</u>-CH-CSO_2), 7.51 (d, 1H, *J* 8, indole C<u>*H*</u>-C-C), 7.34 (d, 1H, *J* 8, indole C<u>*H*</u>-C-NH), 7.14 (t, 1H, *J* 8, indole C<u>*H*</u>-CH-C), 7.05 (t, 1H, *J* 8, indole C<u>*H*</u>-CH-C-C), 6.91 (s, 1H, indole C<u>*H*</u>-NH), 6.41 (t, 1H, *J* 5.5, lactam N<u>*H*</u>-CH_2), 5.68 (t, 1H, *J* 5, N<u>*H*</u>-CH_2), 4.71 (dd, 1H, *J* 11, 5, lactam C<u>*H*</u>), 3.62 (br.t, 2H, *J* 10.5, equatorial C<u>*H*</u>$_2$-N), 3.52 (q, 2H, *J* 6, C<u>*H*</u>$_2$-CH_2-indole), 3.31-3.22 (m, 2H, lactam C<u>*H*</u>$_2$-NH), 2.90 (t, 2H, *J* 6.5, C<u>*H*</u>$_2$-indole), 2.31 (br. t, 2H, *J* 10.5, axial C<u>*H*</u>$_2$-N), 2.14 (d, 1H, *J* 14, equatorial lactam C<u>*H*</u>$_2$-CH)

2.05-1.99 (m, 2H, lactam C*H*$_2$-CH$_2$CH), 1.93-1.79 (m, 3H, C*H*-CH$_2$CH$_2$N and lactam C*H*$_2$-CH$_2$NH),), 1.75-1.61 (m, 4H, C*H*$_2$-CH$_2$N), 1.56 (q, 1H, *J* 12, axial lactam C*H*$_2$-CH) and 1.38 (q, 1H, *J* 12.5, equatorial lactam C*H*$_2$-CH$_2$NH); δ_C (100 MHz, $CDCl_3$) 175.5, 173.6, 164.8 (C=O), 137.1, 136.5 (*C*-CO and *C*-SO_2), 135.4 (indole *C*-NH), 131.3 (*C*H-CCO or *C*H-CSO_2), 130.4 (*C*H-CCO or *C*H-CSO_2), 129.5 (*C*H-CH-CSO_2), 127.3 (indole *C*-C-NH), 126.2 (OCC-*C*H-SO_2), 122.2 (indole *C*H-NH), 122.1 (indole *C*H-C-C), 119.4 (indole *C*H-CH-C), 118.6 (indole *C*H-CH-C-C), 112.6 (indole *C*-CH_2), 111.5 (indole *C*H-C-NH), 52.8 (lactam *C*H), 45.4 (*C*H_2-N), 42.2 (lactam *C*H_2-NH), 41.4 (*C*H-CH_2-CH_2N), 39.6 (*C*H_2-CH_2-indole), 31.4 (lactam *C*H_2-CH), 28.8, 28.0 (lactam *C*H_2-CH_2NH and *C*H_2-CH_2N), 27.9 (*C*H_2-indole) and 25.1 (lactam *C*H_2-CH_2CH); ESI *m/*z 100 %, 588.1 (MNa^+); HR ESI *m/*z ($C_{29}H_{35}N_5O_5SNa^+$ requires 588.2251) found 588.2264.

2.85, (*S*)-*N*-(2-(1*H*-indol-3-yl)ethyl)-1-(4-(2-oxoazepan-3-ylcarbamoyl) phenylsulfonyl)piperidine-4-carboxamide

Acid **2.83** (2.88 g, 6.80 mmol) was dissolved in dichloromethane (50 mL) and cooled to 0° C. HATU (2.51 g, 6.60 mmol) was added and the mixture was stirred for 4 hours. Tryptamine (1.09 g, 6.80 mmol) and triethylamine (2.9 mL, 20 mmol) were added and the reaction was stirred for 48 hours. The dichloromethane was removed *in vacuo* and the product was dissolved in ethyl acetate and MeOH and was washed with a pH 2 buffer (3 × 25 mL) and ½ saturated $NaHCO_3$ (3 × 25 mL). The organic layer was reduced *in vacuo* and the product was purified by silica column chromatography (petroleum ether:ethyl acetate:MeOH 50:50:0 to 0:90:10) to give compound **2.85** as a white solid (2.69 g, 70 %); *mp* 135-136 °C; $[\alpha]^{29}_D$ (c = 0.104, MeOH) + 11.06.υ_{max}/cm^{-1}: 3248 (N-H), 2930 (saturated C-H), 1644 (C=O amide), 1544 (aromatic), 1338, 1154 (SO_2); δ_H (300 MHz, $CDCl_3$) 10.77 (br.s, 1H, N*H*

indole), 8.54 (d, 1H, *J* 7, N*H*-CH), 8.10 (d, 1H, *J* 8.5, C*H*-CO), 7.89-7.80 (m, 4H, C*H*-CSO_2, lactam N*H*-CH_2 and N*H*-CH_2), 7.50 (d, 1H, *J* 8, indole C*H*-C-C), 7.32 (d, 1H, *J* 8, indole C*H*-C-NH), 7.10 (d, 1H, *J* 2, indole C*H*-NH), 7.05 (t, 1H, *J* 7.5, indole C*H*-CH-C), 6.96 (t, 1H, *J* 7.5, indole C*H*-CH-C-C), 4.71 (dd, 1H, *J* 10, 7.5, lactam C*H*), 3.62 (d, 2H, *J* 11.5, equatorial CH_2-N), 3.32-3.20 (m, 3H, lactam CH_2-NH and CH_2-CH_2-indole), 3.14-3.06 (m, 1H, lactam CH_2-NH), 2.77 (t, 2H, *J* 7.5, CH_2-indole), 2.31 (br.t, 2H, *J* 11.5, axial CH_2-N), 2.10 (tt, 1H, *J* 11, 4, C*H*-CH_2CH_2N), 1.92 (t, 2H, *J* 11.5, equatorial lactam CH_2-CH and CH_2-CH_2CH), 1.82-1.68 (m, 4H, equatorial CH_2-CH_2N, equatorial lactam CH_2-CH_2NH and axial CH_2-CH_2CH), 1.57 (br.t, 3H, *J* 11.5, axial CH_2-CH_2N and axial lactam CH_2-CH) and 1.25 (q, 1H, *J* 12, axial lactam CH_2-CH_2NH); δ_C (100 MHz, $CDCl_3$) 174.0, 173.2, 164.1 (C=O), 138.2, 137.8, 136.2 (*C*-SO_2, *C*-CO, indole *C*-NH), 128.3 (*C*H-CCO), 127.5 (*C*H-CSO_2), 127.2 (indole *C*-C-NH), 122.6 (indole *C*H-NH), 120.9 (indole *C*H-C-C), 118.2 (indole *C*H-CH-C), 118.1 (indole *C*H-CH-C-C), 111.7 (indole *C*-CH_2), 111.3 (indole *C*H-C-NH), 52.2 (lactam *C*H), 45.3 (*C*H_2-N), 40.6 (lactam *C*H_2-NH), 40.3 (*C*H-CH_2CH_2N), 40.1 (*C*H_2-CH_2-indole), 30.5 (lactam *C*H_2-CH), 28.9, 27.8 (lactam *C*H_2-CH_2NH and *C*H_2-CH_2N), 27.7 (*C*H_2-indole) and 25.1 (lactam *C*H_2-CH_2CH); ESI *m/*z 100 %, 588.2 (MNa^+) and 13 %, 565.1 (MH^+); HR ESI *m/*z ($C_{29}H_{35}N_5O_5SNa^+$ requires 588.2251) found 588.2263.

2.07, *N*-((*R*)-3-(1*H*-indol-3-yl)-1-oxo-1-(propylamino)propan-2-yl)-1-(3-((*S*)-2-oxoazepan-3-ylcarbamoyl)phenylsulfonyl)piperidine-4-carboxamide

Acid **2.09** (1.27 g, 3.00 mmol) was dissolved in dichloromethane (30 mL) and cooled to 0° C. HATU (1.10 g, 2.89 mmol) was added and the mixture was stirred for 4 hours. Amine **2.27** (2.80 mmol) and triethylamine (1.2 mL, 2.9 mmol) were added and the reaction was stirred for 48 hours. The dichloromethane was removed

in vacuo and the product was dissolved in ethyl acetate and washed with a pH 2 buffer (3 × 15 mL), 0.1 M HCl (3 × 15 mL) and ½ saturated $NaHCO_3$ (3 × 15 mL). The organic layer was reduced *in vacuo* and the product was purified by silica column chromatography (petroleum ether:ethyl acetate:MeOH 40:60:0 to 0:70:30) to give compound **2.07** as a white solid (1.04 g, 57 %) *mp* 147-149 °C; $[\alpha]^{24}_{D}$ (c = 0.099, MeOH) + 9.34; υ_{max}/cm^{-1}: 3272 (N-H), 2929 (saturated C-H), 1639 (C=O amide), 1515 (aromatic) and 1334, 1129 (SO_2); δ_H (400 MHz, $CDCl_3$) 8.86 (br.s, 1H, N*H*-indole), 8.19 (s, 1H, OCC-C*H*-SO_2), 8.02 (d, 1H, *J* 8, C*H*-CCO or C*H*-CSO_2), 7.94 (d, 1H, *J* 6.5, lactam N*H*-CH), 7.83 (d, 1H, *J* 8, C*H*-CCO or C*H*-CSO_2), 7.57 (t, 1H, *J* 8, C*H*-CH-CSO_2), 7.53 (d, 1H, *J* 8, indole C*H*-C-C), 7.29 (d, 1H, *J* 8, indole C*H*-C-NH), 7.10 (t, 1H, *J* 7.5, indole C*H*-CH-C), 7.00 (t, 1H, *J* 7.5, indole C*H*-CH-C-C), 6.92 (br.s, 2H, indole CH-N*H* and lactam N*H*-CH_2), 6.84 (d, 1H, *J* 8, N*H*-CH-CH_2-indole), 6.37 (t, 1H, *J* 6, N*H*-^{n}Pr), 4.74 (dd, 1H, *J* 11.5, 6, lactam C*H*), 4.68 (q, 1H, *J* 7.5, C*H*-CH_2-indole), 3.61 (d, 1H, *J* 11.5, C*H*$_2$-N), 3.48 (d, 1H, *J* 11.5, C*H*$_2$-N), 3.33-3.25 (m, 2H, lactam C*H*$_2$-NH), 3.17-2.97 (m, 4H , C*H*$_2$-indole and C*H*$_2$-NH), 2.32-2.14 (m, 2H, C*H*$_2$-N and equatorial lactam C*H*$_2$-CH), 2.15-1.98 (m, 2H, axial lactam C*H*$_2$-CH_2NH and C*H*$_2$-CH_2CH), 1.92-1.78 (m, C*H*-CH-CH_2N and equatorial lactam C*H*$_2$-CH_2CH), 1.68-1.61 (m, 2H, C*H*$_2$-CH_2N), 1.58-1.50 (m, 3H, C*H*$_2$-CH_2N and axial lactam C*H*$_2$-CH), 1.40 (br.q, 1H, equatorial lactam C*H*$_2$-CH_2NH), 1.29 (sextet, 2H, *J* 7.5, C*H*$_2$-CH_3) and 0.72 (t, 2H, *J* 7.5, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 175.5, 173.8, 171.6, 164.8 (C=O), 137.1 ,136.2, 135.4 (*C*-SO_2, *C*-CO and indole *C*-NH), 131.3 (*C*H-CCO), 130.3 (*C*H-CSO_2), 129.5 (*C*H-CH-CSO_2), 127.4 (indole *C*-C-NH), 126.2 (OCC-*C*H-SO_2), 123.1 (indole *C*H-NH), 122.1 (indole *C*H-C-C), 119.5 (indole *C*H-CH-C), 118.7 (indole *C*H-CH-C-C), 111.4 (indole *C*H-C-NH), 110.6 (indole *C*-CH_2), 53.9 (*C*H-CH_2-indole), 52.9 (lactam *C*H),

45.3 (<u>*C*</u>H_2-N), 42.1 (lactam <u>*C*</u>H_2-NH), 41.4 (<u>*C*</u>H-CH_2CH_2N), 41.2 (<u>*C*</u>H_2-CH_2CH_3), 31.3 (lactam <u>*C*</u>H_2-CH), 28.8 (<u>*C*</u>H_2-indole), 28.4, 28.0 (<u>*C*</u>H_2-CH_2N and lactam <u>*C*</u>H_2-CH_2NH), 27.6 (lactam <u>*C*</u>H_2-CH_2CH), 22.5 (<u>*C*</u>H_2-CH_3), 11.2 (<u>*C*</u>H_3); ESI *m/*z 100 %, 673.2 (MNa^+); HR ESI *m/*z ($C_{33}H_{42}N_6O_6SNa^+$ requires 673.2779) found 673.2774.

2.86, *N*-((*R*)-3-(1*H*-indol-3-yl)-1-oxo-1-(propylamino)propan-2-yl)-1-(4-((*S*)-2-oxoazepan-3-ylcarbamoyl)phenylsulfonyl)piperidine-4-carboxamide

Acid **2.83** (4.00 mmol) was dissolved in dichloromethane (60 mL) and cooled to 0° C. HATU (1.67 g, 4.39 mmol) was added and the mixture was stirred for 4 hours. Amine **2.27** (7 mmol) and triethylamine (1.7 mL, 4.0 mmol) were added and the reaction was stirred for 48 hours. The dichloromethane was removed *in vacuo* and the product was dissolved in ethyl acetate and washed with a pH 2 buffer (3 × 15 mL) and ½ saturated $NaHCO_3$ (3 × 15 mL). The organic layer was reduced *in vacuo* and the product was purified by silica column chromatography (petroleum ether:ethyl acetate:MeOH 25:75:0 to 0:70:30) and then by recrystallisation from ethyl acetate and petroleum ether to give compound **2.86** as a white solid (1.23 g, 47 %); *mp* 143-144 °C; $[\alpha]^{28}_D$ (c = 0.2, MeOH) + 12.31; υ_{max}/cm^{-1}: 3287 (N-H), 2926 (saturated C-H), 1640 (C=O amide), 1516 (aromatic) and 1335, 1166 (SO_2); δ_H (500 MHz, d^6-DMSO) 10.75 (d, 1H, *J* 1.5, N<u>*H*</u>-indole), 8.54 (d, 1H, *J* 7, lactam N<u>*H*</u>-CH), 8.07 (d, 2H, *J* 8, C<u>*H*</u>-CCO), 7.88 (t, 1H, *J* 6, lactam N<u>*H*</u>-CH_2 or N<u>*H*</u>-^{n}Pr), 7.87-7.84 (m, 2H, N<u>*H*</u>-$CHCH_2$-indole and lactam N<u>*H*</u>-CH_2 or N<u>*H*</u>-^{n}Pr), 7.80 (d, 2H, *J* 8, C<u>*H*</u>-CSO_2), 7.55 (d, 1H, *J* 8, indole C<u>*H*</u>-C-C), 7.31 (d, 1H, *J* 8.5, indole C<u>*H*</u>-C-NH), 7.05 (s, 1H, indole C<u>*H*</u>-NH), 7.04 (t, 1H, *J* 8, indole C<u>*H*</u>-CH-C), 6.94 (t, 1H, *J* 8, indole C<u>*H*</u>-CH-C-C), 4.65 (dd, 1H, *J* 10, 7.5, lactam C<u>*H*</u>), 4.45 (q, 1H, *J* 7.5, C<u>*H*</u>-CH_2-indole), 3.53 (d, 1H, *J* 11.5, equatorial C<u>*H*</u>$_2$-N), 3.47-3.40 (m, 1H, equatorial C<u>*H*</u>$_2$-N), 3.25 (td, 1H, *J* 13, 5, axial lactam C<u>*H*</u>$_2$-NH), 3.14-3.07 (m, 1H, equatorial lactam

C<u>*H*</u>$_2$-NH), 3.03 (dd, 1H, *J* 14.5, 5, C<u>*H*</u>$_2$-indole), 3.01-2.90 (m, 2H, C<u>*H*</u>$_2$-Et), 2.87 (dd, 1H, *J* 14.5, 8.5, C<u>*H*</u>$_2$-indole), 2.32 (t, 1H, *J* 10.5, axial C<u>*H*</u>$_2$-N), 2.28 (t, 1H, *J* 10.5, axial C<u>*H*</u>$_2$-N), 2.15 (tt, 1H, *J* 10.5, 4, C<u>*H*</u>-CH_2CH_2N,), 1.91 (br.t, 2H, *J* 14, lactam C<u>*H*</u>$_2$-CH and C<u>*H*</u>$_2$-CH_2CH), 1.80-1.76 (m, 1H, equatorial lactam C<u>*H*</u>$_2$-CH_2NH), 1.70 (br.t, 2H, *J* 13, lactam C<u>*H*</u>$_2$-CH and C<u>*H*</u>$_2$-CH_2CH), 1.63-1.55 (m, 2H, equatorial C<u>*H*</u>$_2$-CH_2N), 1.50 (q, 1H, *J* 12.5, axial C<u>*H*</u>$_2$-CH_2N), 1.48-1.39 (m, 1H, axial C<u>*H*</u>$_2$-CH_2N), 1.32 (sextet, 2H, *J* 7.5, C<u>*H*</u>$_2$-CH_3), 1.25 (br.q, 1H, *J* 12.5, axial lactam C<u>*H*</u>$_2$-CH_2NH), and 0.75 (t, 2H, *J* 7.5, C<u>*H*</u>$_3$); δ_C (125 MHz, d^6-DMSO) 174.5, 173.6, 171.8,164.6 (C=O), 138.7, 138.3, 136.5 (<u>*C*</u>-SO_2, <u>*C*</u>-CO and indole <u>*C*</u>-NH), 128.7 (<u>*C*</u>H-CCO), 127.9 (<u>*C*</u>H-CSO_2), 127.8 (indole <u>*C*</u>-C-NH), 124.0 (indole <u>*C*</u>H-NH), 121.3 (indole <u>*C*</u>H-CH-C), 119.0 (indole <u>*C*</u>H-C-C), 118.6 (indole <u>*C*</u>H-CH-C-C), 111.7 (indole <u>*C*</u>H-C-NH), 110.6 (indole <u>*C*</u>-CH_2), 53.7 (<u>*C*</u>H-CH_2-indole), 52.7 (indole <u>*C*</u>H), 45.6 (<u>*C*</u>H_2-N), 41.3 (lactam <u>*C*</u>H_2-NH), 41.2 (<u>*C*</u>H_2-CH_2CH_3), 40.1 (<u>*C*</u>H-CH_2CH_2N), 31.0 (lactam <u>*C*</u>H_2-CH), 29.4 (<u>*C*</u>H_2-indole), 28.6, 28.2 (<u>*C*</u>H_2-CH_2N and lactam <u>*C*</u>H_2-CH_2NH), 28.2 (lactam <u>*C*</u>H_2-CH_2CH), 22.6 (<u>*C*</u>H_2-CH_3), 11.7 (<u>*C*</u>H_3); ESI *m/*z 100 %, 673.2 (MNa^+); HR ESI *m/*z ($C_{33}H_{42}N_6O_6SH^+$ requires 651.2959) found 651.2965.

2.87, *N*-((*R*)-3-(1*H*-indol-3-yl)-1-oxo-1-(propylamino)propan-2-yl)-1-(3-((*S*)-2-oxopiperidin-3-ylcarbamoyl)phenylsulfonyl)piperidine-4-carboxamide

Acid **2.84** (2.21 g, 5.40 mmol) was dissolved in dichloromethane (50 mL) and cooled to 0° C. HATU (2.01 g, 5.29 mmol) was added and the mixture was stirred for 4 hours. Amine **2.27** (7 mmol) and triethylamine (2.3 mL, 5.4 mmol) were added and the reaction was stirred for 48 hours. The dichloromethane was removed *in vacuo* and the product was dissolved in ethyl acetate and MeOH and washed with a pH 2 buffer (3 × 15 mL). The organic layer was reduced *in vacuo* and the product was purified by silica column chromatography (petroleum ether:ethyl acetate:MeOH

50:50:0 to 0:70:30) and then by washing with ethyl acetate to give compound **2.87** as a white solid (0.82 g, 24 %); *mp* 167-168 °C; $[\alpha]^{20}_{D}$ (c = 0.52, MeOH) -1.49; υ_{max}/cm^{-1}: 3280 (N-H), 2935 (saturated C-H), 1634 (C=O amide), 1543 (aromatic) 1328, 1147 (SO_2); δ_H (500 MHz, d^6-DMSO) 10.74 (d, 1H, *J* 2, N*H* indole), 8.99 (d, 1H, *J* 8.5, lactam N*H*-CH), 8.20-8.18 (m, 2H, OCC-C*H*-SO_2 and C*H*-CCO or C*H*-CSO_2), 7.88-7.86 (m, 2H, N*H*-CH_2-CH_2-indole and N*H*-Pr), 7.84 (d, 1H, *J* 8, C*H*-CCO or C*H*-CSO_2), 7.75 (t, 1H, *J* 8, C*H*-CH-CSO_2), 7.69 (br.s, 1H, lactam N*H*-CH_2), 7.55 (d, 1H, *J* 8, indole C*H*-C-C), 7.30 (d, 1H, *J* 8.5, indole C*H*-C-NH). 7.05 (s, 1H, indole C*H*-NH), 7.04 (t, 1H, *J* 7.5, indole C*H*-CH-C-C), 6.94 (t, 1H, *J* 7.5, indole C*H*-CH-C), 4.44 (dd, 1H, *J* 14, 8.5, C*H*-CH_2-indole), 4.42 (m, 1H, lactam C*H*), 3.58 (d, 1H, *J* 12, equatorial C*H*$_2$-N), 3.47 (d, 1H, *J* 12, equatorial C*H*$_2$-N), 3.21-3.17 (m, 2H, lactam C*H*$_2$-NH), 3.04 (dd, 1H, *J* 14, 6, C*H*$_2$-indole), 3.01-2.91 (m, 2H, C*H*$_2$-Et), 2.87 (dd, 1H, *J* 14, 8.5, C*H*$_2$-indole), 2.29 (td, 1H,*J* 12, 3.5, axial C*H*$_2$-N), 2.24 (td, 1H, *J* 12, 3.5, axial C*H*$_2$-N), 2.15 (tt, 1H, *J* 11.5, 4, C*H*-CH_2CH_2N), 2.04-1.99 (m, 1H, lactam C*H*$_2$-CH), 1.87-1.78 (m, 3H, lactam C*H*$_2$-CH and C*H*$_2$-CH_2NH), 1.70 (br.d, 1H, *J* 12.5, equatorial C*H*$_2$-CH_2-N), 1.58 (br.d, 1H, *J* 12.5, equatorial C*H*$_2$-CH_2-N), 1.50 (qd, 1H, *J* 12.5, 4.5, axial C*H*$_2$-CH_2N), 1.38 (qd, 1H, *J* 12.5, 4.5, axial C*H*$_2$-CH_2N), 1.28 (sextet, 2H, *J* 7.5, C*H*$_2$-CH_3) and 0.76 (t, 3H, *J* 7.5, C*H*$_3$); δ_C (125 MHz, d^6-DMSO) 173.7, 171.8,170.0, 164.7 (C=O), 136.5, 136.3, 135.6 (*C*-SO_2, *C*-CO and indole *C*-NH), 132.3, 130.5 (*C*H-CCO and *C*H-CSO_2), 130.2 (*C*H-CH-CSO_2), 127.8 (indole *C*-C-NH), 126.4 (OCC-*C*H-SO_2), 124.0 (indole *C*H-NH), 121.3 (indole *C*H-CH-C-C), 119.0 (indole *C*H-C-C), 118.9 (indole *C*H-CH-C), 111.9 (indole *C*H-C-NH), 110.6 (indole *C*-CH_2), 53.7 (*C*H-CH_2-indole), 50.1 (lactam *C*H), 45.7 (*C*H_2-N), 41.7 (lactam *C*H_2-NH), 40.8 (*C*H_2-Et), 40.3 (C*H*-CH_2CH_2N), 28.6 (lactam *C*H_2-CH), 28.2 (*C*H_2-indole), 28.1 (*C*H_2-CH_2N), 27.8

(lactam <u>C</u>H_2-CH_2NH), 22.8 (<u>C</u>H_2-CH_3) and 11.9 (<u>C</u>H_3); ESI *m/*z 100 %, 659.2 (MNa^+) and 20 %, 636.5 (MH^+); HR ESI *m/*z ($C_{32}H_{40}N_6O_6SNa^+$ requires 659.2622) found 659.2635.

2.88, *O*-Benzyl 4-((*R*)-3-(1*H*-indol-3-yl)-2-(1-(3-((*S*)-2-oxoazepan-3-ylcarbamoyl) phenylsulfonyl)piperidine-4-carboxamido)propanamido)butylcarbamate

Acid **2.09** (0.26 g, 0.61 mmol) was dissolved in dichloromethane (10 mL) and cooled to 0° C. HATU (0.33 g, 0.87 mmol) was added and the mixture was stirred for 4 hours. Amine **2.28** (0.75 mmol) and triethylamine (0.30 mL, 2.3 mmol) were added and the reaction was stirred for 48 hours. The dichloromethane was removed *in vacuo* and the product was dissolved in ethyl acetate and was washed with a pH 2 buffer (3 × 10 mL), 0.1 M HCl (3 × 10 mL) and ½ saturated $NaHCO_3$ (3 × 10 mL). The organic layer was reduced *in vacuo* and the product was purified by silica column chromatography (petroleum ether:ethyl acetate:MeOH 50:50:0 to 0:90:10) to give compound **2.88** as a light yellow solid (0.39 g, 79 %); *mp* 110-111° C; $[\alpha]^{29}_D$ (c = 0.2, MeOH) + 5.13; δ_H (700 MHz, DMSO) 10.73 (s, 1H, N<u>H</u> indole), 8.69 (d, 1H, *J* 7, lactam N<u>H</u>-CH), 8.20 (d, 1H, *J* 8, C<u>H</u>-CCO or C<u>H</u>-CSO_2), 8.18 (s, 1H, OCC-C<u>H</u>-SO_2), 7.89 (t, 1H, *J* 5.5, N<u>H</u>-$CHCH_2$-indole), 7.85-7.83 (m, 3H, C<u>H</u>-CCO or C<u>H</u>-CSO_2, N<u>H</u>-Cbz and lactam N<u>H</u>-CH_2), 7.75 (t, 1H, *J* 8, C<u>H</u>-CH-CSO_2), 7.55 (d, 1H, *J* 8, indole C<u>H</u>-C-C), 7.36-7.28 (m, 6H, indole C<u>H</u>-C-NH and phenyl), 7.20 (t, 1H, *J* 5.5, N<u>H</u>-CH_2CH_2), 7.05 (s, 1H, indole C<u>H</u>-NH), 7.04 (t, 1H, *J* 7.5, indole C<u>H</u>-CH-C), 6.94 (t, 1H, *J* 7.5, indole C<u>H</u>-CH-C-C), 4.99 (s, 2H, C<u>H_2</u>-phenyl), 4.67 (dd, 1H, *J* 11, 8, lactam C<u>H</u>), 4.44 (q, 1H, *J* 7.5, C<u>H</u>-CH_2-indole), 3.56 (d, 1H, *J* 12, equatorial C<u>H_2</u>-N), 3.47 (d, 1H, *J* 12, equatorial C<u>H_2</u>-N), 3.24 (td, 1H, *J* 13.5, 5, C<u>H_2</u>-NHCO), 3.10 (m, 1H, C<u>H_2</u>-NHCO), 3.03 (dd, 1H, *J* 14.5, 5.5, C<u>H_2</u>-indole), 2.99-2.92 (m, 4H, lactam C<u>H_2</u>-NH and C<u>H_2</u>-NHCbz), 2.86 (dd, 1H, *J* 14.5, 8, C<u>H_2</u>-

indole), 2.28 (t, 1H, *J* 11, axial C$\underline{H_2}$-N), 2.24 (t, 1H, *J* 11, axial C$\underline{H_2}$-N), 2.14 (tt, 1H, *J* 10.5, 3.5, C$\underline{H}$-CH_2CH_2N), 1.93 (br.d, 1H *J* 14, equatorial lactam C$\underline{H_2}$-CH), 1.88 (br.d, 1H, *J* 13.5, equatorial lactam C$\underline{H_2}$-CH_2NH), 1.71-1.66 (m, 2H, lactam C$\underline{H_2}$-CH_2CH), 1.67 (t, 1H, *J* 12.5, axial lactam C$\underline{H_2}$-CH), 1.57 (br.d, 2H, *J* 12, equatorial C$\underline{H_2}$-CH_2N), 1.45 (br.q, 1H, *J* 12, axial C$\underline{H_2}$-CH_2N) 1.37 (br.q, 1H, *J* 12, axial C$\underline{H_2}$-CH_2N) and 1.25 (br.q, 1H, *J* 12.5, axial lactam C$\underline{H_2}$-CH_2NH); δ_C (100 MHz, d^6 DMSO) 169.1, 168.2, 166.1, 158.7, (C=O amide), 150.9 (C=O carbamate), 131.4, 130.6, 130.5, 129.5 (*C*-SO_2, *C*-CONH, indole and *C*-NH, *C* phenyl), 125.9, 124.4 (*C*H-CCO and *C*H-CSO_2), 124.0 (*C*H-CH-CSO_2), 122.7 (OCC-*C*H-SO_2), 122.1, 122.1 (phenyl *C*H), 121.7 (indole *C*-C-NH), 120.6 (indole *C*H-NH), 117.9 (indole *C*H-CH-C-C), 115.3 (indole *C*H-C-C), 112.8 (indole *C*H-CH-C), 105.7 (indole *C*H-C-NH), 104.3 (indole *C*-CH_2), 59.9 (*C*H_2-phenyl), 48.1 (*C*H-CH_2-indole), 46.8 (lactam *C*H), 39.7 (*C*H_2-N), 35.5 (lactam *C*H_2-NH), 34.8 (*C*H-CH_2CH_2N), 34.5, 33.0, 32.9, 25.1 (lactam *C*H_2-CH, *C*H_2-indole, *C*H_2-NHCbz and *C*H_2-NHCO), 23.1, 22.3, 22.2 (lactam *C*H_2-CH_2CH lactam *C*H_2-CH_2NH and *C*H_2-CH_2-N) and 21.8, 21.1 (*C*H_2-CH_2-NHCbz and *C*H_2-CH_2NHCO); ESI *m/*z ($C_{42}H_{51}N_7O_8SNa^+$ requires 836.3418) found 836.2.

2.89, *O*-Benzyl 5-((*R*)-3-(1*H*-indol-3-yl)-2-(1-(3-((*S*)-2-oxoazepan-3-ylcarbamoyl) phenylsulfonyl) piperidine-4-carboxamido)propanamido)pentylcarbamate

Acid **2.09** (1.47 g, 3.48 mmol) was dissolved in dichloromethane (40 mL) and cooled to 0° C. HATU (1.29 g, 3.39 mmol) was added and the mixture was stirred for 4 hours. Amine **2.29** (3.4 mmol) and triethylamine (1.40 mL, 10.2 mmol) were added and the reaction was stirred over night. The dichloromethane was removed *in vacuo* and the product was dissolved in ethyl acetate and washed with a pH 2 buffer (3 × 15 mL), 0.1 M HCl (3 × 15 mL) and ½ saturated $NaHCO_3$ (3 × 15 mL). The

organic layer was dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate:MeOH , 50:50:0 to 0:10:90) to give compound **2.89** as a white-yellow solid (0.36 g, 13 %); *mp* 118-120 ^{o} C; $[\alpha]^{22}_{D}$ = 53.35 MeOH; υ_{max}/cm^{-1}: 3299 (N-H), 2930 (saturated C-H), 1641 (C=O amide), 1516 (aromatic), 1335, 1149 (SO_2) and 1249 (C-O); δ_H (700 MHz, d^6-DMSO) 10.72 (d, 1H, *J* 2.5, N*H* indole), 8.69 (d, 1H, *J* 7.5, lactam N*H*-CH), 8.19 (t, 1H, *J* 8, C*H*-CH-CSO_2), 8.18 (s, 1H, OCC-C*H*-CSO_2), 7.88-7.82 (m, 4H, C*H*-CCO, C*H*-CSO_2, lactam N*H*-CH_2 and N*H*-CH-CH_2-indole), 7.75 (t, 1H, *J* 7.5, N*H*Cbz or N*H*-pentyl), 7.55 (d, 1H, *J* 7.5, indole C*H*-C-C), 7.37-7.28 (m, 6H, indole C*H*-C-C and phenyl), 7.20 (t, 1H, *J* 5.5, , N*H*Cbz or N*H*-pentyl), 7.05 (d, 1H, *J* 2.5, indole C*H*-NH), 7.03 (d, 1H, *J* 7.5, indole C*H*-CH-C-C), 6.94 (t, 1H, *J* 7.5, indole C*H*-CH-C), 4.99 (s, 2H, C*H*$_2$-phenyl), 4.66 (ddd, 1H, *H* 11.5, 8, 1.5, lactam C*H*), 4.44 (q, 1H, *J* 8.5, C*H*-CH_2-indole), 3.59 (d, 1H, *J* 10.5, equatorial C*H*$_2$-N), 3.48 (d, 1H, *J* 10.5, equatorial C*H*$_2$-N), 3.24 (td, 1H, *J* 13, 5, axial lactam C*H*$_2$-NH), 3.13-3.08 (m, 1H, equatorial lactam C*H*$_2$-NH), 3.03 (dd, 1H, *J* 14.5, 5, C*H*$_2$-indole), 2.99 (t, 1H, *J* 6.5, C*H*$_2$-NHCbz) and 2.97-2.92 (m, 3H, C*H*$_2$-NHCbz and C*H*$_2$-butyl), 2.86 (dd, 1H, *J* 14.5, 8.5, C*H*$_2$-indole), 2.29 (td, 1H, *J* 11.5, 2.5, axial C*H*$_2$-N), 2.24 (td, 1H, *J* 11.5, 2.5, axial C*H*$_2$-N), 2.14 (tt, 1H, *J* 11, 4, C*H*-CH_2CH_2N), 1.93 (d, 1H, *J* 14, equatorial lactam C*H*$_2$-CH), 1.88 (d, 1H, *J* 14, lactam C*H*$_2$-CH_2NH), 1.81-1.77 (m, 2H, C*H*$_2$-CH_2NHCbz), 1.72-1.66 (m, 2H, lactam C*H*$_2$-CH_2NH and C*H*$_2$-propyl), 1.62 (td, 1H, *J* 12, 2, C*H*$_2$-CH_2CH), 1.58 (dd, 1H, *J* 16, 2.5, lactam C*H*$_2$-CH_2CH), 1.50 (qd, 1H, *J* 12, 3.5, axial lactam C*H*$_2$-CH), 1.42-1.22 (m, 4H, C*H*$_2$-CH_2N), 1.18-1.12 (m, 2H, C*H*$_2$-CH_2CH_2NHCbz); δ_C (175 MHz, d^6-DMSO) 174.6, 173.6, 171.7, 164.3 (C=O), 156.5 (C=O carbamate), 137.7, 136.4, 136.39, 135.7 (*C*-SO_2, *C*-CONH, phenyl *C* and indole *C*-NH), 132.2 , 130.4 (*C*H-CCO and *C*H-CSO_2), 130.1 (*C*H-CH-CSO_2),

128.8 (OCC-<u>C</u>H-CSO_2), 128.2 ,126.5 (phenyl), 127.8 (indole <u>C</u>-C-NH), 123.9 (indole <u>C</u>H-NH), 121.2 (indole <u>C</u>H-CH-C-C), 118.9 (indole <u>C</u>H-C-C), 118.5 (indole <u>C</u>H-CH-C), 111.6 (indole <u>C</u>H-C-NH), 110.6 (indole <u>C</u>-CH_2), 65.5 (<u>C</u>H_2-phenyl), 53.7 (<u>C</u>H-CH_2-indole), 52.7 (lactam <u>C</u>H), 45.5 (<u>C</u>H_2-NSO_2), 41.2 (lactam <u>C</u>H_2-NH), 40.7, 39.6 (<u>C</u>H_2-butyl and <u>C</u>H_2-NHCbz), 40.0 (<u>C</u>H-CH_2CH_2N), 31.0 (<u>C</u>H_2-CH_2N), 29.5, 29.4, 29.0, 28.5, 28.2, 27.9 (<u>C</u>H_2-indole, lactam <u>C</u>H_2-CH, <u>C</u>H_2-CH_2NH, <u>C</u>H_2-CH_2CH and C<u>*H*</u>$_2$-CH_2NHCbz, C<u>*H*</u>$_2$-propyl) and 24.0 (<u>C</u>H_2-CH_2CH_2NHCbz); ESI *m/*z (100 %, 850.5 (MNa^+).

2.90, *O*-Benzyl 6-((*R*)-3-(1*H*-indol-3-yl)-2-(1-(4-((*S*)-2-oxoazepan-3-ylcarbamoyl) phenylsulfonyl)piperidine-4-carboxamido)propanamido)hexylcarbamate

Acid **2.84** (0.061 g, 0.160 mmol) was dissolved in dichloromethane (5 mL) and cooled to 0°C. HATU (0.08 g, 0.21 mmol) was added and the mixture was stirred for 4 hours. Amine **2.30** (0.16 mmol) and triethylamine (67 µL, 0.48 mmol) were added and the reaction was stirred over night. The dichloromethane was removed *in vacuo* and the product was dissolved in ethyl acetate and washed with a pH 2 buffer (3 × 5 mL) and ½ saturated $NaHCO_3$ (3 × 5 mL). The organic layer was dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate:MeOH, 50:50:0 to 0:90:10) to give compound **2.90** as a white solid (0.047 g, 36 %); *mp* 54-57 º C; δ_H (700 MHz, $CDCl_3$) 8.07 (d, 2H, *J* 8.5, C<u>*H*</u>-CCO), 7.83 (d, 2H, *J* 8.5, C<u>*H*</u>-CSO_2), 7.57 (d, 1H, *J* 8.5, indole C<u>*H*</u>-C-C), 7.36-7.27 (m, 6H, indole C<u>*H*</u>-C-NH and phenyl), 7.09 (1H, t, *J* 7.5, indole C<u>*H*</u>-CH-C), 7.06 (s, 1H, indole C<u>*H*</u>-NH), 6.99 (t, 1H, *J* 7.5, indole C<u>*H*</u>-CH-C-C), 5.08 (s, 2H, C<u>*H*</u>$_2$-phenyl), 4.81 (dd, 1H, *J* 10, 1.5, lactam C<u>*H*</u>), 4.60 (t, 1H, *J* 7.5, C<u>*H*</u>-CH_2-indole), 3.69 (d, 1H, *J* 11.5, equatorial C<u>*H*</u>$_2$-N), 3.60 (d, 1H, *J* 11.5, equatorial C<u>*H*</u>$_2$-N), 3.38 (dd, 1H, *J* 15, 11.5, axial lactam C<u>*H*</u>$_2$-NH), 3.28 (dd, 1H, *J* 14.5, 5,

equatorial lactam C$\underline{H_2}$-NH), 3.20 (dd, 1H, *J* 14.5, 7, C$\underline{H_2}$-indole), 3.13-3.04 (m, 4H, C$\underline{H_2}$-NHCbz and C$\underline{H_2}$-pentyl), 3.00 (dd, 1H, *J* 14.5, 7, C$\underline{H_2}$-indole), 2.34 (td, 1H, *J* 12, 3, axial C$\underline{H_2}$-N), 2.30 (td, 1H, *J* 12, 3, axial C$\underline{H_2}$-N), 2.14 (tt, 1H, *J* 1H, C$\underline{H}$-CH_2CH_2N), 2.10-2.04 (m, 2H, C$\underline{H_2}$-CH and C$\underline{H_2}$-CH_2NH), 1.92-1.84 (m, 2H, C$\underline{H_2}$-CH_2CH), 1.78-1.67 (m, 2H, C$\underline{H_2}$-CH_2NHCbz), 1.63-1.59 (m, 1H, lactam C$\underline{H_2}$-CH), 1.58-1.52 (m, 1H, lactam C$\underline{H_2}$-CH_2NH), 1.45-1.39 (m, 4H, C$\underline{H_2}$-CH_2CH_2N), 1.31-1.27 (m, 2H, C$\underline{H_2}$-butyl), 1.25-1.21 (m, 2H, C$\underline{H_2}$-$CH_2CH_2CH_2NHCbz$) and 1.14-1.09 (m, 2H, C$\underline{H_2}$-CH_2CH_2NHCbz); δ_C (175 MHz, $CDCl_3$) 175.6, 175.1, 172.4, 166.0 (C=O), 157.5 (C=O carbamate), 138.8, 138.1, 137.1, 136.6 (*C*-SO_2, *C*-CO, indole *C*-NH and phenyl *C*), 128.1 (*C*H-CCO), 128.0 (*C*H-CSO_2), 127.5, 127.4 (phenyl), 127.3 (indole *C*-C-NH), 123,1 (indole *C*H-NH), 121.0 (indole *C*H-CH-C), 118.5 (indole *C*H-CH-C-C), 118.4 (indole *C*H-C-C), 111.1 (indole *C*H-C-NH), 109.6 (indole *C*-CH_2), 65.9 (*C*H_2-phenyl), 54.5 (*C*H-CH_2-indole), 52.7 (lactam *C*H), 45.4 (*C*H_2-N), 41.2 (lactam *C*H_2-NH), 41.1 (*C*H-CH_2CH_2N), 38.9, 38.8 (*C*H_2-NHCbz and *C*H_2-pentyl), 30.7 (lactam *C*H_2-CH), 29.4, 29.3, 28.6, 28.56, 28.53, 27.9, 27.8, 27.5 8 (lactam *C*H_2-CH_2CH, lactam *C*H_2-CH_2NH, *C*H_2-*C*H_2-*C*H_2-*C*H_2-CH_2NHCbz, *C*H_2-indole and *C*H_2-CH_2N.

2.91, (*S*)-*tert*-butyl 2-((*R*)-3-(1*H*-indol-3-yl)-2-(1-(3-((*S*)-2-oxoazepan-3-ylcarbamoyl)phenylsulfonyl)piperidine-4-carboxamido)propanamido)-6-(benzyloxycarbonylamino)hexanoate

Acid **2.09** (2.0 g, 4.7 mmol) was dissolved in dichloromethane (35 mL) and cooled to 0° C. HATU (1.52 g, 4.00 mmol) was added and the mixture was stirred for 4 hours. Amine **2.31** (4 mmol) and triethylamine (1.7 mL, 4.0 mmol) were added and the reaction was stirred for 48 hours. The dichloromethane was removed *in vacuo* and the product was dissolved in ethyl acetate and washed with a pH 2 buffer (3 × 20

mL), 0.1 M HCl (3 × 20 mL) and ½ saturated $NaHCO_3$ (3 × 20 mL). The organic layer was reduced *in vacuo* and the product was purified by silica column chromatography (petroleum ether:ethyl acetate:MeOH 50:50:0 to 0:90:10) to give compound **2.91** as a white solid (1.32 g, 71 %); *mp* 127-128 °C; $[\alpha]^{22}_{D}$ = -7.08 MeOH; υ_{max}/cm^{-1}: 3284 (N-H), 2932 (saturated C-H), 1705 (C=O ester), 1643 (C=O amide), 1513 (aromatic), 1334, 1150 (SO_2) and 1250 (C-O); δ_H (700 MHz, $CDCl_3$) 9.33 (d, 1H, N*H* indole), 8.17 (s, 1H, OCC-C*H*-CSO_2), 8.00 (d, 1H, *J* 7.5, C*H*-CCO or C*H*-CSO_2), 7.83 (d, 1H, *J* 7.5, C*H*-CCO or C*H*-CSO_2), 7.80 (d, 1H, *J* 5.5, lactam N*H*-CH), 7.63 (d, 1H, *J* 8, indole C*H*-C-C), 7.55 (t, 1H, *J* 8, C*H*-CH-CSO_2), 7.33-7.27 (m, 5H, indole *C*H-C-NH and phenyl), 7.10 (t, 1H, *J* 8, indole *C*H-CH-C), 7.04 (t, 1H, *J* 7.5, indole *C*H-CH-C-C), 6.90 (s, 1H, indole *C*H-NH), 6.60 (br.t, 1H, *J* 5, lactam N*H*-CH_2), 6.50 (d, 1H, *J* 7, N*H*-$CHCH_2$-indole), 6.20 (d, 1H, *J* 7.5, N*H*-CHCOO), 5.10 (d, 1H, *J* 12, C*H*$_2$-phenyl) 5.09 (d, 1H, *J* 5.5, C*H*$_2$-phenyl), 5.06 (t, 1H, *J* 6, N*H*Cbz), 4.75 (q, 1H, *J* 7, C*H*-CH_2-indole), 4.69 (dd, 1H, *J* 11, 5, lactam C*H*), 4.25 (q, 1H, *J* 6.5, C*H*-COO), 3.65 (d, 1H, *J* 11.5, equatorial C*H*$_2$-N), 3.59-3.56 (m, 1H, equatorial), C*H*$_2$-N 3.29 (td, 1H, *J* 11, 5, axial lactam C*H*$_2$-NH), 3.25-3.20 (m, 1H, C*H*$_2$-indole), 3.18 (dd, 1H, *J* 13.5, 5,5, equatorial lactam C*H*$_2$-NH), 3.10-3.01 (m, 2H, C*H*$_2$-indole and C*H*$_2$-NHCbz), 2.96 (qu, 1H, *J* 6, C*H*$_2$-NHCbz), 2.36-2.28 (m, 2H, axial C*H*$_2$-N), 2.18 (d, 1H, *J* 13.5, axial C*H*$_2$-N), 2.05-2.00 (m, 2H, lactam C*H*$_2$-CH and C*H*$_2$-CH_2NH), 1.98-1.92 (m, 1H, C*H*-$CH_2CH_2NSO_2$), 1.84 (br.d, 2H, *J* 11.5, C*H*$_2$-CHCOO), 1.77-1.65 (m, 4H, C*H*$_2$-CH_2N), 1.55 (q, 1H, *J* 11.5, axial lactam C*H*$_2$-CH), 1.47-1.41 (m, 1H, lactam C*H*$_2$-CH_2CH), 1.39 (d, 1H, *J* 13, lactam C*H*$_2$-CH_2CH), 1.36-1.30 (m, 2H, lactam C*H*$_2$-CH_2NH and C*H*$_2$-CH_2NHCbz), 1.33 (s, 9H, C(C*H*$_3$)$_3$), 1.29-1.24 (m, 1H, C*H*$_2$-CH_2NHCbz), 0.84-0.77 (m, 1H, C*H*$_2$-CH_2CHCOO) and 0.75-0.69 (m, 1H, C*H*$_2$-CH_2CHCOO); δ_C (700 MHz, $CDCl_3$)

175.4 (C=O ester), 173.4, 170.9, 170.7, 164.7 (C=O), 156.8 (C=O carbamate), 137.2, 136.5, 136.4, 135.4 (*C*-SO_2, *C*-CONH, indole *C*-NH and phenyl *C*), 131.2, 130.3 (*C*H-CCO and *C*H-CSO_2), 129.4 (*C*H-CH-CSO_2), 128.6 (OCC-*C*H-SO_2), 128.2 (phenyl *m*-*C*H), 128.1 (phenyl *p*-*C*H), 127.2 (indole *C*-C-NH), 126.2 (phenyl *o*-*C*H), 123.1 (indole *C*H-NH), 122.1 (indole *C*H-CH-C), 119.6 (indole *C*H-CH-C-C), 118.7 (indole *C*H-C-C), 111.5 (indole *C*H-C-NH), 110.1 (indole *C*-CH_2), 82.2 (*C*$(CH_3)_3$), 66.8 (C*H*$_2$-phenyl), 53.8 (*C*-CH_2-indole), 52.8 (*C*-COO), 52.4 (lactam *C*H), 45.4 (*C*H_2-N), 42.2 (lactam *C*H_2-NH), 41.6 (*C*H-CH_2CH_2N), 40.8 (*C*H_2-NHCbz), 31.8 (*C*H_2-CH_2N), 31.4, 29.8, 29.0, 28.8, 27.9, 27.8, (*C*H_2-indole, lactam *C*H_2-CH, lactam *C*H_2-CH_2NH, lactam *C*H_2-CH_2CH, *C*H_2-CHCOO and *C*H_2CH_2NHCbz), 28.0 (C(*C*$H_3)_3$) and 21.6 (*C*H_2-CH_2CHCOO); HR ESI *m/*z ($C_{48}H_{61}N_7O_{10}SH^+$ requires 928.4273) found 928.4284.

2.92, (*S*)-*tert*-Butyl 2-((*R*)-3-(1*H*-indol-3-yl)-2-(1-(4-((S)-2-oxoazepan-3-ylcarbamoyl)phenylsulfonyl)piperidine-4-carboxamido)propanamido)-6-(benzyloxycarbonylamino)hexanoate

Acid **2.84** (0.81 g, 1.9 mmol) was dissolved in dichloromethane and the reaction was cooled to 0 °C. HATU (0.79 g, 2.1 mmol) was added and the reaction was stirred for 10 minutes. Amine **2.31** (2 mmol) in dichloromethane and triethylamine (0.9 mL, 6 mmol) were added and the reaction stirred overnight. The dichloromethane was removed *in vacuo* and the product was dissolved in ethyl acetate and washed with a pH 2 buffer (3 × 20 mL) and ½ saturated $NaHCO_3$ (3 × 20 mL). The organic layer was dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate:MeOH 60:40:0 to 0:70:30) to give compound **2.92** as a white powder (1.36 g, 76 %); δ_H (700 MHz, MeOD) 8.08 (d, 2H, *J* 8, C*H*-CCO), 7.85 (d, 2H, *J* 8, C*H*-CSO_2), 7.59 (d, 1H, *J* 7.5, indole

C*H*-C-C), 7.38-7.31 (m, 5H, phenyl), 7.11 (t, 1H, *J* 8, indole C*H*-CH-C), 7.08 (s, 1H, indole C*H*-NH), 7.02 (t, 1H, *J* 7.5, indole C*H*-CH-C-C), 5.08 (s, 2H, CH_2-phenyl), 4.83 (d, 1H, *J* 11.5 lactam C*H*), 4.75 (t, 1H, *J* 7.5, C*H*-CH_2-indole), 4.11 (dd, 1H, *J* 8, 5, C*H*-COO), 3.72 (d, 1H, *J* 12.5, equatorial CH_2-N), 3.64 (d, 1H, *J* 12.5, equatorial CH_2-N), 3.39 (t, 1H, *J* 13.5 axial lactam CH_2-NH), 3.29 (dd, 1H, *J* 14, 4, equatorial lactam CH_2-NH), 3.22 (dd, 1H, *J* 14, 7.5, CH_2-indole), 3.09 (dd, 1H, *J* 14, 7, CH_2-indole), 3.06-3.02 (m, 2H, CH_2-NHCbz), 2.36 (t, 1H, *J* 11.5, axial CH_2-N), 2.32 (t, 1H, *J* 11.5, axial CH_2-N), 2.16 (tt, 1H, *J* 11, 2.5, C*H*-CH_2CH_2N), 2.08 (br.d, 2H, *J* 11.5, lactam CH_2-CH and lactam CH_2-CH_2NH), 1.93-1.85 (m, 2H, lactam CH_2-CH_2CH), 1.80-1.68 (m, 4H, CH_2-CH_2N and CH_2CHCOO), 1.65-1.56 (m, 2H, lactam CH_2-CH and lactam CH_2-CH_2NH), 1.47-1.43 (m, 2H, CH_2-CH_2N), 1.44 (s, 9H, C(CH_3)$_3$), 1.40-1.34 (m, 2H, CH_2-CH_2NHCbz) and 1.05-0.99 (m, 2H, CH_2-CH_2CH-COO); δ_C (175 MHz, MeOD) 175.6 (C=O ester), 175.0, 172.3, 171.2, 166.1 (C=O amide), 157.5 (C=O carbamate), 138.9, 138.1, 137, 136.5 (*C*-SO_2, *C*-CONH, indole *C*-NH and phenyl *C*), 128.1 (*C*H-CCO), 127.5 (*C*H-CSO_2), 127.9, 127.6 (phenyl), 127.3 (indole *C*-C-NH), 123.2 (indole *C*H-NH), 121.2 (indole *C*H-CH-C-C), 118.5 (indole *C*H-C-C), 118.1 (indole *C*H-CH-C), 111.1 (indole *C*H-C-NH), 109.6 (indole *C*-CH_2), 81.5 (*C*(CH_3)$_3$), 65.9 (CH_2-phenyl), 55.0 (*C*-CH_2-indole), 54.0 (*C*-COO^{t}Bu), 53.0 (lactam *C*H), 45.4 (*C*H_2-N), 41.3 (lactam *C*H_2-NH), 41.2 (*C*H-CH_2CH_2N), 41.1 (*C*H_2-NHCbz), 30.8 (*C*H_2-CH_2N), 30.0, 29.2, 29.1, 29.0, 28.9, 28.6 (*C*H_2-indole, lactam *C*H_2-CH, lactam *C*H_2-CH_2NH, lactam *C*H_2CH_2CH, *C*H_2-CH-COO^{t}Bu and *C*H_2-CH_2-NH-Cbz), 27.0 (C(*C*H_3)$_3$) and 22.5 (*C*H_2-CH_2CHCOO); HR ESI *m/*z ($C_{48}H_{60}N_7O_{10}SH^+$ requires 927.4201) found 928.4273 and ($C_{48}H_{61}N_7O_{10}SNa^+$ requires 950.4098) found 950.4093.

2.06, *N*-((*R*)-1-(5-Aminopentylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)-1-(3-((*S*)-2-oxoazepan-3-ylcarbamoyl)phenylsulfonyl)piperidine-4-carboxamide

Carbamate **2.89** (0.23 g, 0.27 mmol) was dissolved in MeOH (8 mL) and THF (2 mL). Palladium activated charcoal (0.5 g, catalytic) was added and the reaction stirred under hydrogen for 24 hours. The palladium was removed through filtration and the organic solvent was removed *in vacuo* to give compound **2.06** as a brown solid (0.16 g, 81 %); $[\alpha]^{24}_{D}$ (c = 0.0858, MeOH) +4.56; δ_H (400 MHz, MeOD) 8.53 (s, 1H, OCC-C*H*-CSO_2), 8.18 (d, 1H, *J* 7.5, C*H*-CCO or C*H*-CSO_2), 7.93 (d, 1H, *J* 8, C*H*-CCO or C*H*-CSO_2), 7.74 (t, 1H, *J* 8, C*H*-CH-CSO_2), 7.59 (d, 1H, *J* 8, indole C*H*-C-C), 7.37 (d, 1H, *J* 8.5, indole C*H*-C-NH), 7.12 (s, 1H, indole C*H*-NH), 7.11 (t, 1H, *J* 7.5, indole C*H*-CH-C), 7.02 (t, 1H, *J* 7.5, indole C*H*-CH-C-C), 4.84 (d, 1H, *J* 9, lactam C*H*), 4.58 (t, 1H, *J* 8.5, C*H*-CH_2-indole), 3.74 (d, 1H, *J* 11.5, equatorial CH_2-N), 3.65 (d, 1H, *J* 11.5, equatorial CH_2-N), 3.39-3.31 (m, 2H, lactam CH_2-NH), 3.25 (dd, 1H, *J* 14.5, 7.5, CH_2-indole), 3.16-3.00 (m, 3H, CH_2-indole and CH_2-NHCO), 2.87 (dd, 1H, *J* 8, 8.5, CH_2-NH_2), 2.37 (td, 1H, *J* 12, 3, axial CH_2-N), 2.32 (td, 1H, *J* 12, 3, axial CH_2-N), 2.13 (m, 1H, C*H*-CH_2CH_2N), 2.08 (br.d, 2H, *J* 11, lactam CH_2-CH), 1.90 (td, 2H, *J* 12, 4, CH_2-CH_2N), 1.84-1.58 (m, 6H, lactam CH_2-CH_2CH, lactam CH_2-CH_2NH and CH_2-CH_2N), 1.43-1.34 (m, 4H, CH_2-CH_2NH_2 and CH_2-CH_2NHCO) and 1.27-1.20 (m, 2H, CH_2-$CH_2CH_2NH_2$); δ_C (175 MHz, MeOD) 175.7, 175.4, 172.7, 165.9 (C=O), 136.8, 136.6, 135.2 (*C*-SO_2, *C*-CONH and indole *C*-NH), 131.3 (*C*H-CCO or *C*H-CSO_2), 130.3 (*C*H-CCO or *C*H-CSO_2), 129.4 (*C*H-CH-CSO_2), 127.4 (indole *C*-C-NH), 126.4 (OCC-*C*H-CSO_2), 123.2 (indole *C*H-NH), 121.1 (indole *C*H-CH-C-C), 118.4 (indole *C*H-C-C), 118.0 (indole *C*H-CH-C), 110.9 (indole *C*H-C-NH), 109.6 (indole *C*-CH_2), 54.5 (*C*H-CH_2-indole), 52.8 (lactam *C*H), 45.4 (*C*H_2-N), 41.1 (lactam *C*H_2-NH), 41.0 (*C*H-CH_2-CH_2N), 39.2 (*C*H_2-NH_2), 38.5

($\underline{C}H_2$-NHCO), 30.5 ($\underline{C}H_2$-CH_2N), 28.6, 28.3, 28.1, 28.0, 27.9, 27.7 ($\underline{C}H_2$-indole, lactam $\underline{C}H_2$-CH, lactam $\underline{C}H_2$-CH_2NH, lactam $\underline{C}H_2$-CH_2CH and $\underline{C}H_2$-CH_2-$\underline{C}H_2CH_2NH_2$), 23.0 ($\underline{C}H_2$-$CH_2CH_2NH_2$); HR ESI *m/z* ($C_{35}H_{48}N_7O_6SH^+$ requires 694.3381) found 694.3398.

2.93, *N*-((*R*)-1-(6-Aminohexylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)-1-(4-((*S*)-2-oxoazepan-3-ylcarbamoyl)phenylsulfonyl)piperidine-4-carboxamide

Carbamate **2.90** (0.047 g, 0.056 mmol) was dissolved in MeOH (4 mL), palladium activated charcoal (0.01 g, catalytic) was added and the reaction stirred under hydrogen over night. The palladium was removed through filtration and the organic solvents removed *in vacuo* to give compound **2.93** as a light brown solid (0.02 g, 50 %); δ_H (400 MHz, MeOD) 8.10 (d, 2H, *J* 8.5, C$\underline{H}$-CCO), 7.87 (d, 2H, *J* 8.5, C$\underline{H}$-CSO_2), 7.60 (d, 1H, *J* 7.5, indole C$\underline{H}$-C-C), 7.36 (d, 1H, *J* 8, indole C$\underline{H}$-C-NH), 7.12 (1H, t, *J* 7.5, indole C$\underline{H}$-CH-C), 7.09 (s, 1H, indole C$\underline{H}$-NH), 7.03 (t, 1H, *J* 7.5, indole C$\underline{H}$-CH-C-C), 4.87 (d, 1H, *J* 11, lactam C$\underline{H}$), 4.60 (t, 1H, *J* 7, C$\underline{H}$-CH_2-indole), 3.74 (d, 1H, *J* 12, equatorial C$\underline{H}_2$-N), 3.64 (d, 1H, *J* 12, equatorial C$\underline{H}_2$-N), 3.45-3.36 (m, 2H, lactam C$\underline{H}_2$-NH), 3.22 (dd, 1H, *J* 14, 7, C$\underline{H}_2$-indole), 3.17-3.03 (m, 3H, C$\underline{H}_2$-indole and C$\underline{H}_2$-pentyl), 2.72 (t, 2H, *J* 8, C$\underline{H}_2$-NH_2), 2.39 (td, 1H, *J* 10, 3, axial C$\underline{H}_2$-N), 2.34 (td, 1H, *J* 10, 3, axial C$\underline{H}_2$-N), 2.22-2.15 (m, 1H, *J* 1H, C$\underline{H}$-CH_2CH_2N), 2.09 (br.d, 1H, *J* 10.5, equatorial C$\underline{H}_2$-CH_2N), 1.97-1.887 (m, 2H, C$\underline{H}_2$-CH_2NH_2), 1.85-1.45 (m, 8H, axial C$\underline{H}_2$-CH_2N, lactam C$\underline{H}_2$-CH_2NH, lactam C$\underline{H}_2$-CH_2CH, lactam C$\underline{H}_2$-CH), 1.40-1.27 (4H, C$\underline{H}_2$-C$\underline{H}_2$-CH_2NHCO) and 1.23-1.15 (m, 2H, C$\underline{H}_2$-$CH_2CH_2NH_2$); HR ESI ($C_{36}H_{50}N_7O_6S$ requires 708.3538) found 708.3536.

2.05, (*S*)-*tert*-Butyl-2-((*R*)-3-(1*H*-indol-3-yl)-2-(1-(3-((*S*)-2-oxoazepan-3-ylcarbamoyl)phenylsulfonyl)piperidine-4-carboxamido)propanamido)-6-aminohexanoate

Carbamte **2.91** (0.5 g, 0.5 mmol) was dissolved in MeOH (4 mL) and THF (2 mL). Palladium activated charcoal (0.2 g, catalytic) was added and the reaction stirred under hydrogen for 24 hours. The palladium activated charcoal was removed through filtration and the organic solvent was removed *in vacuo* to give compound **2.05** as a white solid (0.18 g, 45 %); *mp* 140-145 °C (decomposed); $[\alpha]^{24}_{D}$ (c = 0.244, $CHCl_3$) +0.61; υ_{max}/cm^{-1}: 3254 (N-H), 2928 (saturated C-H), 1723 (C=O ester), 1638 (C=O amide), 1544 (aromatic), 1331 (SO_2), 1274 (C-O) and 1150 (SO_2); δ_H (400 MHz, MeOD) 8.25 (s, 1H, OCC-C*H*-CSO_2), 8.15 (d, 1H, *J* 7.5, C*H*-CCO or C*H*-CSO_2), 7.90 (d, 1H, *J* 8.5, C*H*-CCO or C*H*-CSO_2), 7.69 (t, 1H, *J* 7.5, C*H*-CH-CSO_2), 7.58 (d, 1H, *J* 8, indole C*H*-C-C), 7.34 (d, 1H, *J* 8.5, indole C*H*-C-NH), 7.10 (s, 1H, indole C*H*-NH), 7.09 (t, 1H, *J* 7.5, indole C*H*-CH-C), 7.0 (t, 1H, *J* 7.5, indole C*H*-CHC-C), 4.85-4.81 (m, 1H, C*H*-COO^tBu), 4.74 (t, 1H, *J* 7.5, C*H*-CH_2-indole), 4.10 (dd, 1H, *J* 9.5, 5, lactam C*H*), 3.72 (d, 1H, *J* 12.5, quatorialCH_2-N e), 3.64 (d, 1H, *J* 12.5, equatorial CH_2-N), 3.35-3.32 (m, 2H, lactam CH_2-NH), 3.19 (dd, 1H, *J* 14, 7.5, CH_2-indole), 3.05 (dd, 1H, *J* 14, 7.5, CH_2-indole), 2.65 (t, 2H, *J* 8, CH_2-NH_2), 2.44-2.33 (m, 2H, axial CH_2-N), 2.13 (tt, 1H, *J* 11, 3.5, C*H*-CH_2CH_2N), 2.03 (br.d, 2H, *J* 11.5, lactam CH_2-CH and CH_2-CH_2NH), 1.88-1.80 (m, 2H, CH_2-CH_2N), 1.77-1.65 (m, 2H, CH_2-CH_2N), 1.62-1.52 (m, 4H, lactam CH_2-CH_2CH and CH_2-CH-COO), 1.49-1.45 (m, 2H, lactam CH_2-CH), 1.43 (s, 9H, $C(CH_3)_3$), 1.31 (quintet, 2H, CH_2-CH_2NH_2) and 0.98 (quintet, 2H, *J* 7.5, CH_2-CH_2CH and CH_2-CH_2NH); δ_C (175 MHz, MeOD) 175.8, 175.1, 172.4, 171.3, 165.8 (C=O), 136.8 136.6 135.2 (*C*-SO_2, *C*-CONH and indole *C*-NH), 131.3, 130.2 (*C*H-CCO or *C*H-CSO_2), 129.4 (*C*H-CH-

CSO_2), 127.5 (indole <u>C</u>-C-NH), 126.4 (OCC-<u>C</u>H-CSO_2), 123.2 (indole <u>C</u>H-NH), 121.1 (indole <u>C</u>H-CH-C-C), 118.4 (indole <u>C</u>H-C-C), 118.1 (indole <u>C</u>H-CH-C), 111.0 (indole <u>C</u>H-C-NH), 109.6 (indole <u>C</u>-CH_2), 81.3 (<u>C</u>$(CH_3)_3$), 54.0 (<u>C</u>H-CH_2-indole), 53.2 (<u>C</u>H-COO), 52.7 (lactam <u>C</u>H), 45.4 (<u>C</u>H_2-N), 41.1 (lactam <u>C</u>H_2-NH), 41.08 (<u>C</u>H-CH_2-CH_2N), 40.8 (<u>C</u>H_2-NH_2), 31.8 (<u>C</u>H_2-CH_2N), 30.6 (<u>C</u>H_2-CH-COO), 28.69, 28.1, 27.9, 27.8, 27.7 (lactam <u>C</u>H_2-CH, <u>C</u>H_2CH_2CH and <u>C</u>H_2CH_2NH, <u>C</u>H_2-indole, <u>C</u>H_2-CH_2NH_2), 26.9 (C(<u>C</u>$H_3)_3$), 22.4 (<u>C</u>H_2-CH_2CHCOO); HR ESI ($C_{40}H_{55}N_7O_8SH^+$ requires 794.3906) found 794.3915.

2.94, (*S*)-*tert*-Butyl 2-((*R*)-3-(1*H*-indol-3-yl)-2-(1-(4-((*S*)-2-oxoazepan-3-ylcarbamoyl)phenylsulfonyl)piperidine-4-carboxamido)propanamido)-6-aminohexanoate

Carbamate **2.92** (0.70 g, 0.75 mmol) was dissolved in MeOH (8 mL). Palladium activated charcoal (0.3 g, catalytic) was added and the reaction stirred under hydrogen for 24 hours. The palladium was removed through filtration and the organic solvent was removed *in vacuo* to give compound **2.94** as a beige oil (0.40 g, 67 %); υ_{max}/cm^{-1}: 3273 (N-H), 2931 (saturated C-H), 1725 (C=O ester), 1638 (C=O amide), 1535 (aromatic), 1332 (SO_2), 1250 (C-O) and 1154 (SO_2); δ_H (700 MHz, MeOD) 8.09 (d, 2H, *J* 8.5, C<u>H</u>-CCO), 7.87 (d, 2H, *J* 8.5, C<u>H</u>-CSO_2), 7.59 (d, 1H, *J* 8, indole C<u>H</u>-C-C), 7.35 (d, 1H, *J* 8, indole C<u>H</u>-C-NH), 7.11 (t, 1H, *J* 7.5, indole C<u>H</u>-CH-C), 7.10 (s, 1H, indole C<u>H</u>-NH), 7.02 (t, 1H, *J* 7.5, indole C<u>H</u>-CH-C-C), 4.83 (d, 1H, *J* 11, C<u>H</u>-COO), 4.71 (t, 1H, *J* 7.5, C<u>H</u>-CH_2-indole), 4.24 (dd, 1H, *J* 8.5, 5, lactam C<u>H</u>), 3.74 (d, 1H, *J* 12, equatorial CH_2-N), 3.66 (d, 1H, *J* 12, equatorial CH_2-N), 3.41-3.27 (m, 2H, lactam C<u>H</u>$_2$-NH), 3.22 (dd, 1H, *J* 14, 7.5, C<u>H</u>$_2$-indole), 3.08 (dd, 1H, *J* 14, 7.5, C<u>H</u>$_2$-indole), 2.57 (t, 2H, *J* 7.5, C<u>H</u>$_2$-NH_2), 2.44-2.33 (m, 2H, axial C<u>H</u>$_2$-N), 2.20 (tt, 1H, *J* 10.5, 4, C<u>H</u>-CH_2CH_2N), 2.14-20.5 (br.d, 2H, equatorial

C<u>*H*</u>$_2$-CH_2N), 1.99-1.79 (m, 3H, lactam C<u>*H*</u>$_2$-CH_2NH, lactam C<u>*H*</u>$_2$-CH, lactam C<u>*H*</u>$_2$-CH_2CH), 1.74 (q, 2H, *J* 14, axial C<u>*H*</u>$_2$-CH_2-N), 1.67-1.56 (m, 4H, lactam C<u>*H*</u>$_2$-CH_2NH, lactam C<u>*H*</u>$_2$-CH, lactam C<u>*H*</u>$_2$-CH_2CH and C<u>*H*</u>$_2$-CH-COO), 1.44 (m, 10H, C<u>*H*</u>$_2$-CHCOO and C(C<u>*H*</u>$_3$)$_3$), 1.40-1.31 (m, 2H, C<u>*H*</u>$_2$-CH_2NH_2), 1.00 (quin., 2H, *J* 7.5, C<u>*H*</u>$_2$-CH_2CHCOO); δ_C (175 MHz, MeOD) 175.6 (C=O ester), 175.0, 172.4, 171.3, 165.8 (C=O), 138.8, 138.2, 136.5 (<u>*C*</u>-SO_2, C-CO and indole <u>*C*</u>-NH), 128.1 (<u>*C*</u>H-CCO), 127.6 (<u>*C*</u>H-CSO_2), 127.5 (indole <u>*C*</u>-C-NH), 123.4 (indole <u>*C*</u>H-NH), 121.2 (indole <u>*C*</u>H-CH-C-C), 118.5 (indole <u>*C*</u>H-C-C), 118.2 (indole <u>*C*</u>H-CH-C), 111.1 (indole <u>*C*</u>H-C-NH), 109.7 (indole <u>*C*</u>-CH_2), 81.4 (<u>*C*</u>(CH_3)$_3$), 54.0 (<u>*C*</u>H-CH_2-indole), 53.2 (<u>*C*</u>H-COO), 52.7 (lactam <u>*C*</u>H), 45.3 (<u>*C*</u>H_2-N), 41.2 (lactam <u>*C*</u>H_2-NH), 41.0 (<u>*C*</u>H-CH_2CH_2N), 40.7 (<u>*C*</u>H_2-NH_2), 31.7 (<u>*C*</u>H_2-CH_2N), 30.7, 28.6, 27.9, 27.7, 27.3, 27.0, (<u>*C*</u>H_2-indole, lactam <u>*C*</u>H_2-CH, lactam <u>*C*</u>H_2-CH_2NH, lactam <u>*C*</u>H_2-CH_2CH, <u>*C*</u>H_2-CH_2NH_2 and <u>*C*</u>H_2-CHCOO), 27.0 (C(<u>*C*</u>H_3)$_3$) and 22.4 (<u>*C*</u>H_2-CH_2CHCOO); HR ESI *m/*z ($C_{40}H_{55}N_7O_8SH^+$ requires 794.3906) found 794.3921.

6.3 Chapter 3 Experimental

6.3.1 Lactams with long Alkyl Chains

3.33, Decanoylamino-(*S*)-tetrahydropyridin-2-one

Lactam **2.53** (20 mmol) was dissolved in H_2O (40 mL) and cooled to 0°C. Decanoyl chloride (3.1 mL, 15 mmol) in dichloromethane (40 mL) and triethylamine (6.3 mL, 45 mmol) were added and stirred over night. The reaction was extracted with dichloromethane (3 × 30 mL), the organic layer was washed with a pH 2 buffer (3 × 20 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 50:50:0 to 0:80:20) to give compound **3.33** as a white solid 1.22 g (30 %); *mp* 109-110 ° C; $[\alpha]^{27}_D$ (c = 0.26,

$CHCl_3$) +75.05; υ_{max}/cm^{-1}: 3202 (N-H), 2922 (saturated C-H) and 1656, 1638 (C=O); *Anal.* ($C_{15}H_{28}N_2O_2$ requires: C; 67.13, H; 10.52, N; 10.44) found: C; 66.87, H; 10.41, N; 10.26; δ_H (400 MHz, $CDCl_3$) 7.21 (s, 1H, N*H*-CH_2), 6.91 (d, 1H, *J* 6.5, N*H*-CH), 4.11 (dt, 1H, *J* 6, 11.5, C*H*), 3.18-3.12 (m, 2H, lactam C*H*$_2$-NH), 2.29-2.12 (m, 1H, lactam C*H*$_2$-CH), 2.05 (t, 2H, *J* 7.5, C*H*$_2$-CO), 1.76-1.70 (m, 2H, lactam C*H*$_2$-CH_2NH), 1.51-1.41 (m, 3H, lactam C*H*$_2$-CH and C*H*$_2$-CH_2CO), 1.17-1.08 (m, 10H, C*H*$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$-CH_3) and 0.71 (t, 3H, *J* 7.5, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 173.5, 172.2 (*C*=O), 50.1 (*C*H), 41.4 (lactam *C*H_2-NH), 36.3 (*C*H_2-CO), 31.8 (lactam *C*H_2-CH), 29.3 (*C*H_2-CH_2CO), 29.2 (*C*H_2-CH_2CH_2CO), 29.1 (*C*H_2-$CH_2CH_2CH_2$CO), 29.0 (*C*H_2-$CH_2CH_2CH_2CH_3$), 27.2 (*C*H_2-$CH_2CH_2CH_3$), 25.6 (C*H*$_2$-CH_2CH_3), 22.5 (C*H*$_2$-CH_3), 21.0 (lactam *C*H_2-CH_2NH) and 14.0 (C*H*$_3$); ESI *m/*z 100 %, 559.3 (M_2Na^+) and 15 %, 269.1 (MH^+); HR ESI *m/*z ($C_{15}H_{28}N_2O_2H^+$ requires 291.2043) found 291.2040.

3.34, Nonanoylamino-(*S*)-tetrahydropyridin-2-one

Lactam **2.53** (20 mmol) was dissolved in H_2O (40 mL) and cooled to 0°C. Nonanoyl chloride (2.8 mL, 15 mmol) in dichloromethane (40 mL) and triethylamine (6.7 mL, 48 mmol) were added and stirred over night. The reaction was extracted with dichloromethane (3 × 30 mL), the organic layer was washed with a pH 2 buffer (3 × 20 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 50:50:0 to 0:80:20) to give compound **3.34** as a white solid 1.04 g (27 %); *mp* 102-103 ° C; $[\alpha]^{27}_D$ (c = 0.244, $CHCl_3$) +86.01; υ_{max}/cm^{-1}: 3304 (N-H), 2922 (saturated C-H) and 1637, 1620 (C=O); *Anal.* ($C_{14}H_{26}N_2O_2$ requires: C; 66.10, H; 10.30, N; 11.01) found: C; 65.96, H; 10.23, N; 10.95; δ_H (400 MHz, $CDCl_3$) 7.14 (s, 1H, N*H*-CH_2), 6.84 (d, 1H, *J* 6, N*H*-CH),

4.13 (dt, 1H, *J* 11.5, 6, C*H*), 3.20-3.14 (m, 2H, lactam C*H*$_2$-NH), 2.30 (ddt, 1H, *J* 5.5, 5, 13, lactam C*H*$_2$-NH), 2.07 (t, 2H, *J* 7.5, C*H*$_2$-CO), 1.79-1.71 (m, 2H, lactam C*H*$_2$-CH$_2$NH), 1.52-1.41 (m, 3H, C*H*$_2$-CH and C*H*$_2$-CH$_2$CO), 1.20-1.07 (C*H*$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$-CH$_3$) and 0.73 (t, 3H, *J* 7.5, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 173.5 (C=O), 172.2 (C=O), 50.1 (*C*H), 41.4 (lactam *C*H$_2$-NH), 36.4 (*C*H$_2$-CO), 31.7 (lactam *C*H$_2$-CH), 29.3 (*C*H$_2$-CH$_2$CO), 29.2 (*C*H$_2$-CH$_2$CH$_2$CO), 29.1 (*C*H$_2$-CH$_2$CH$_2$CH$_2$CO), 27.2 (*C*H$_2$-CH$_2$CH$_2$CH$_3$), 25.7 (*C*H$_2$-CH$_2$CH$_3$), 22.6 (*C*H$_2$-CH$_3$), 21.0 (lactam *C*H$_2$-CH$_2$NH) and 14.0 (*C*H$_3$); ESI *m/*z 100 %, 531.3 (M_2Na^+) and 30 %, 277.1 (MNa^+); HR ESI *m/*z ($C_{14}H_{26}N_2O_2Na$ requires 277.1826) found 277.1884.

3.35, Octanoylamino-(*S*)-tetrahydropyridin-2-one

Lactam **2.53** (20 mmol) was dissolved in H_2O (100 mL) and cooled to 0°C. Octanoyl chloride (2.6 mL, 15 mmol) in dichloromethane (40 mL) and triethylamine (6.7 mL, 48 mmol) were added and stirred over night. The reaction was extracted with dichloromethane (3 × 30 mL), the organic layer was washed with a pH 2 buffer (3 × 20 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 50:50:0 to 0:80:20) to give compound **3.35** as a white solid 0.95 g (26 %); *mp* 106-107 ° C; $[\alpha]^{27}_D$ (c = 0.3, $CHCl_3$) +81.89; υ_{max}/cm^{-1}: 3302, 3201 (N-H), 2924 (saturated C-H), 1670, 1704 (C=O) and 1530 (aromatic); *Anal.* ($C_{13}H_{24}N_2O_2$ requires: C; 64.97, H; 10.07, N; 11.66) found: C; 64.89, H; 10.00, N; 11.47; δ_H (400 MHz, $CDCl_3$) 7.0 (s, 1H, N*H*-CH$_2$), 6.75 (d, 1H, *J* 5.5, N*H*-CH), 4.18 (dt, 1H, *J* 11.5, 5.5, C*H*), 3.25-3.20 (m, 2H, lactam C*H*$_2$-NH), 2.38 (ddt, 1H, *J* 4.5, 6, 13, lactam C*H*$_2$-CH), 2.11 (t, 2H, *J* 8, C*H*$_2$-CO), 1.84-1.76 (m, 2H, lactam C*H*$_2$-CH$_2$NH), 1.56-1.44 (m, 3H, lactam C*H*$_2$-CH and C*H*$_2$-CH$_2$CO), 1.24-1.14 (m, 8H, C*H*$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$-CH$_3$) and 0.77 (t, 3H, *J* 7.5,

C<u>*H*</u>$_3$); δ_C (100 MHz, $CDCl_3$) 173.5, 172.2 (<u>*C*</u>=O), 50.2 (<u>*C*</u>H), 41.5 (lactam <u>*C*</u>H_2-NH), 36.4 (<u>*C*</u>H_2-CO), 31.6 (lactam <u>*C*</u>H_2-CH), 29.2 (<u>*C*</u>H_2-CH_2CO), 29.0 (<u>*C*</u>H_2-CH_2CH_2CO), 25.7 (<u>*C*</u>H_2-$CH_2CH_2CH_3$), 25.6 (<u>*C*</u>H_2-CH_2CH_3), 24.9 (<u>*C*</u>H_2-CH_3), 21.0 (lactam <u>*C*</u>H_2-CH_2CH) and 14.0 (<u>*C*</u>H_3); ESI *m/*z 100 %, 503.0 (M_2Na^+) and 24 %, 263.2 (MNa^+); HR ESI *m/*z ($C_{13}H_{24}N_2O_2Na^+$ requires 263.1730) found 263.1730.

3.36, Heptanoylamino-(*S*)-tetrahydropyridin-2-one

Lactam **2.53** (20 mmol) was dissolved in H_2O (20 mL) and cooled to 0°C. Heptanoyl chloride (2.3 mL, 15 mmol) in dichloromethane (25 mL) and triethylamine (6.3 mL, 45 mmol) were added and stirred over night. The reaction was extracted with dichloromethane (3 × 25 mL), the organic layer was washed with a pH 2 buffer (3 × 20 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 50:50:0 to 0:80:20) to give compound **3.36** as a white solid 0.73 g (21 %); *mp* 101-102 º C; $[\alpha]^{27}_D$ (c = 0.23, $CHCl_3$) +111.52; υ_{max}/cm^{-1}: 3298 (N-H), 2924 (saturated C-H) and 1625 (C=O); *Anal.* ($C_{12}H_{22}N_2O_2$ requires: C; 63.68, H; 9.80, N; 12.38) found: C; 63.38, H; 9.73, N; 12.15; δ_H (400 MHz, $CDCl_3$) 6.66 (br.s, 1H, N<u>*H*</u>-CH_2), 6.60 (d, 1H, *J* 6.5, N<u>*H*</u>-CH), 4.22 (dt, 1H, *J* 12, 5.5, C<u>*H*</u>), 3.30-3.26 (m, 2H, lactam C<u>*H*</u>$_2$-NH), 2.49 (dt, 1H, *J* 12, 5.5, 4.5, lactam equatorial C<u>*H*</u>$_2$-CH), 2.17 (t, 1H, *J* 8, C<u>*H*</u>$_2$-CO), 2.16 (t, 1H, *J* 8, C<u>*H*</u>$_2$-CO), 1.90-1.83 (m, 2H, lactam axial C<u>*H*</u>$_2$-CH_2NH and C<u>*H*</u>$_2$-CH), 1.61-1.44 (m, 3H, lactam axial C<u>*H*</u>$_2$-CH_2NH and C<u>*H*</u>$_2$-CH_2CO), 1.29-1.20 (m, 6H, C<u>*H*</u>$_2$-C<u>*H*</u>$_2$-C<u>*H*</u>$_2$-CH_3) and 0.82 (t, 3H, *J* 7.5, C<u>*H*</u>$_3$); δ_C (100 MHz, $CDCl_3$) 173.5, 172.1 (<u>*C*</u>=O), 50.4 (<u>*C*</u>H), 41.6 (lactam <u>*C*</u>H_2-NH), 36.6 (<u>*C*</u>H_2-CO), 31.5 (lactam <u>*C*</u>H_2-CH), 28.9 (<u>*C*</u>H_2-CH_2CO), 27.2 (<u>*C*</u>H_2-CH_2CH_2CO), 25.6 (<u>*C*</u>H_2-CH_2CH_3), 22.5 (<u>*C*</u>H_2-CH_3), 21.0

(lactam <u>*C*</u>H_2-CH_2NH) and 14.0 (<u>*C*</u>H_3); ESI *m/*z 100 %, 475.2 (M_2Na^+) and 26 %, 249.0 (MNa^+); HR ESI *m/*z ($C_{12}H_{22}N_2O_2Na^+$ requires 249.1573) found 249.1573.

3.37, Hexanoylamino-(*S*)-tetrahydropyridin-2-one

Lactam **2.53** (20 mmol) was dissolved in H_2O (80 mL) and cooled to 0°C. Hexanoyl chloride (2.1 mL, 16 mmol) in dichloromethane (25 mL) and triethylamine (6.3 mL, 48 mmol) were added and stirred over night. The reaction was extracted with dichloromethane (3 × 30 mL), the organic layer was washed with a pH 2 buffer (3 × 20 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 50:50:0 to 0:80:20) to give compound **3.37** as a white solid 0.415 g (13 %); *mp* 101-102 º C; $[\alpha]^{30}_D$ (c = 0.46, $CHCl_3$) +97.39; υ_{max}/cm^{-1}: 3215 (N-H), 2924 (saturated C-H) and 1666 (C=O); *Anal.* ($C_{11}H_{20}N_2O_2$ requires: C; 62.23, H; 9.50, N; 13.20) found: C; 62.08, H; 9.43, N; 12.90; δ_H (400 MHz, $CDCl_3$) 6.52 (d, 1H, *J* 5, N<u>*H*</u>-CH_2), 6.34 (br.s, 1H, N<u>*H*</u>-CH), 4.24 (dt, 1H, *J* 11, 5.5, C<u>*H*</u>), 3.33-3.28 (m, 2H, lactam C<u>*H*</u>$_2$-NH), 2.53 (ddt, 1H, *J* 13, 6, 5, lactam equatorial C<u>*H*</u>$_2$-CH), 2.19 (t, 1H, *J* 8, C<u>*H*</u>$_2$-CO), 2.18 (t, 1H, *J* 8, C<u>*H*</u>$_2$-CO), 1.92-1.86 (m, 2H, lactam C<u>*H*</u>$_2$-CH_2NH), 1.61 (quintet, 2H, *J* 7.5, C<u>*H*</u>$_2$-CH_2CO), 1.32-1.23 (m, 4H, C<u>*H*</u>$_2$-C<u>*H*</u>$_2$-CH_3) and 0.86 (t, 3H, *J* 7.5, C<u>*H*</u>$_3$); δ_C (100 MHz, $CDCl_3$) 173.5, 172.0 (<u>*C*</u>=O), 50.5 (<u>*C*</u>H), 41.7 (lactam <u>*C*</u>H_2-NH), 36.6 (<u>*C*</u>H_2-CO), 31.4 (<u>*C*</u>H_2-CH_2CO), 27.2 (lactam <u>*C*</u>H_2-CH), 25.3 (<u>*C*</u>H_2-CH_2CH_3), 22.4 (<u>*C*</u>H_2-CH_3), 21.0 (lactam <u>*C*</u>H_2-CH_2NH) and 14.0 (<u>*C*</u>H_3); ESI *m/*z 100 %, 447.0 (M_2Na^+) and 33 %, 235.2 (MNa^+); HR ESI *m/*z ($C_{11}H_{20}N_2O_2Na^+$ requires 235.1417) found 235.1419.

3.38, Pentanoylamino-(*R*)-tetrahydropyridin-2-one

Lactam **2.53** (20 mmol) was dissolved in H_2O (20 mL) and cooled to 0°C. Valeryl chloride (1.8 mL, 15 mmol) in dichloromethane (25 mL) and triethylamine (6.3 mL, 45 mmol) were added and stirred over night. The reaction was extracted with dichloromethane (3 × 20 mL), the organic layer was washed with a pH 2 buffer (3 × 20 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 50:50:0 to 0:80:20) to give compound **3.38** as a white solid 0.20 g (10 %); *mp* 87-88 º C; $[\alpha]^{30}_D$ (c = 0.25, $CHCl_3$) -87.72; υ_{max}/cm^{-1}: 3300 (N-H), 2956, 2931 (saturated C-H) and 1622 (C=O); *Anal.* ($C_{10}H_{18}N_2O_2$ requires: C; 60.58, H; 9.15, N; 14.13) found: C; 60.03, H; 9.06, N; 13.55; δ_H (400 MHz, $CDCl_3$) 6.55 (d, 1H, *J* 5, N*H*-CH), 6.46 (br.s, 1H, N*H*-CH_2), 4.24 (dt, 1H, *J* 11.5, 6, C*H*), 3.33-3.28 (m, 2H, lactam C*H*$_2$-NH), 2.52 (ddt, 1H, *J* 13, 5.5, 4, lactam C*H*$_2$-CH), 2.19 (t, 2H, *J* 8, C*H*$_2$-CO), 1.92-1.85 (m, 2H, lactam C*H*$_2$-CH_2NH), 1.59 (quintet, 2H, *J* 7.5, C*H*$_2$-CH_2CO), 1.54-1.45 (m, 1H, lactam C*H*$_2$-CH), 1.31 (sextet, 2H, *J* 7.5, C*H*$_2$-CH_3) and 0.88 (t, 3H, *J* 7, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 173.5 (*C*=O), 172.1 (C=O), 50.5 (*C*H-NH), 41.6 (lactam *C*H_2-NH), 36.3 (*C*H_2-CO), 27.7 (lactam *C*H_2-CH), 27.2 (*C*H_2-CH_2CO), 22.4 (*C*H_2-*C*H_3), 21.0 (lactam *C*H_2-CH_2NH) and 13.8 (*C*H_3); ESI *m/*z 100 %, 419.0 (M_2Na^+) and 26 %, 221.1 (MNa^+); HR ESI *m/*z ($C_{14}H_{18}N_2O_2Na^+$ requires 221.1260) found 221.1252.

3.39, Pentanoylamino-(*S*)-tetrahydropyridin-2-one

Lactam **2.53** (10 mmol) was dissolved in H_2O (15 mL) and cooled to 0°C. Valeryl chloride (1.2 mL, 10 mmol) in dichloromethane (20 mL) and triethylamine (4.2 mL, 30 mmol) were added and stirred over night. The reaction was extracted with dichloromethane (3 × 20 mL), the organic layer was washed with a pH 2 buffer (3 × 20 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica

column chromatography (petroleum ether:ethyl acetate 50:50:0 to 0:80:20) to give compound **3.39** as a white solid 0.13 g (7 %); *mp* 94-95º C; $[\alpha]^{26}_{D}$ (c = 0.491, $CHCl_3$) +97.08; υ_{max}/cm^{-1}: 3290, 3206 (N-H), 2946 (saturated C-H) and 1654, 1638 (C=O); *Anal.* ($C_{10}H_{18}N_2O_2$ requires: C; 60.58, H; 9.15, N; 14.13) found: C; 60.42, H; 9.13, N; 13.96; δ_H (400 MHz, $CDCl_3$) 6.53 (d, 1H, *J* 4, N*H*-CH), 6.38 (br.s, 1H, N*H*-CH_2), 4.24 (dt, 1H, *J* 11.5, 6, C*H*), 3.33-3.29 (m, 2H, lactam C*H*$_2$-NH), 2.53 (ddt, 1H, *J* 12.5, 5.5, 4, lactam C*H*$_2$-CH), 2.20 (t, 2H, *J* 8, C*H*$_2$-CO), 1.93-1.86 (m, 2H, lactam C*H*$_2$-CH_2NH), 1.59 (quintet, 2H, *J* 7.5, C*H*$_2$-CH_2CO), 1.53-1.45 (m, 1H, lactam C*H*$_2$-CH), 1.31 (sextet, 2H, *J* 7.5, C*H*$_2$-CH_3) and 0.88 (m, 3H, *J* 7.5, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 173.5, 172.0 (*C*=O), 50.5 (*C*H), 41.7 (lactam *C*H_2-NH), 36.3 (*C*H_2-CO), 27.7 (lactam *C*H_2-CH), 27.2 (*C*H_2-CH_2CO), 22.4 (*C*H_2-CH_3), 21.0 (lactam *C*H_2-CH_2NH) and 13.8 (*C*H_3); ESI *m/*z 100 %, 419.0 (M_2Na^+) and 17 %, 221.1 (MNa^+); HR ESI *m/*z ($C_{10}H_{18}N_2O_2Na^+$ requires 221.1260) found 221.1262.

3.40, Butanoylamino-(*S*)-tetrahydropyridin-2-one

Lactam **2.53** (10 mmol) was dissolved in H_2O (50 mL) and cooled to 0°C. Butryl chloride (8 mmol) in dichloromethane and triethylamine (3.4 mL, 24 mmol) were added and stirred over night. The reaction was extracted with dichloromethane (3 × 20 mL), the organic layer was washed with a pH 2 buffer (3 × 20 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 50:50:0 to 0:80:20) to give compound **3.40** as a white solid 0.047 g (3 %); *mp* 70-71 º C; $[\alpha]^{27}_{D}$ (c = 0.24, $CHCl_3$) +104.17; υ_{max}/cm^{-1}: 3247 (N-H), 2962 (saturated C-H) and 1649 (C=O); *Anal.* ($C_9H_{16}N_2O_2$ requires: C; 58.67, H; 8.75, N; 15.21) found: C; 55.49, H; 8.40, N; 14.37; δ_H (400 MHz, $CDCl_3$) 6.79 (br.s, 1H, NH-CH_2), 6.68 (d, 1H, *J* 6, N*H*-CH),

4.21 (dt, 1H, *J* 11.5, 5.5, C*H*), 3.30-3.23 (m, 2H, lactam C*H*$_2$-NH), 2.44 (ddt, 1H, *J* 12.5, 5.5, 4, lactam equatorial C*H*$_2$-CH), 2.14 (t, 1H, *J* 7, C*H*$_2$-CO), 2.13 (t, 1H, *J* 7, C*H*$_2$-CO), 1.87-1.81 (m, 2H, lactam C*H*$_2$-CH$_2$NH), 1.60 (sextet, 2H, *J* 7, C*H*$_2$-CH$_2$CO), 1.53-1.44 (m, 1H, lactam C*H*$_2$-CH) and 0.88 (t, 3H, *J* 7.5, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 173.4, 172.2 (C=O), 50.3 (*C*H-NH), 41.5 (lactam *C*H$_2$-NH), 38.4 (*C*H$_2$-CO), 27.3 (lactam *C*H$_2$-CH), 21.0 (*C*H$_2$-CH$_2$CO), 19.0 (lactam *C*H$_2$-CH$_2$NH) and 13.7 (CH_3); ESI *m/*z 100 %, 391.0 (M_2Na^+) and 43 %, 207.1 (MNa^+); HR ESI *m/*z ($C_9H_{16}N_2O_2Na^+$ requires 207.1104) found 207.1105.

6.3.2 6-Membered Exupéry Compounds

3.41, (L)-Orn-(Cbz)-OMe.HCl

Acetyl chloride (5 mL, 70 mmol) was dissolved in MeOH (80 mL) and cooled to 0 °C. (L)-Ornithine-(Cbz) (9.12 g, 34.2 mmol) was added and the reaction was stirred over night, the organic solvents were removed *in vacuo* to give compound **3.41** as a white solid; *mp* 137-138 °C; δ_H (400 MHz, MeOD) 7.39-7.30 (m, 5H, phenyl), 5.10 (s, 2H, C*H*$_2$-phenyl), 4.10 (t, 1H, *J* 6, C*H*), 3.86 (s, 3H, OC*H*$_3$), 3.20 (m, 2H, *J* 7, C*H*$_2$-NH), 2.0-1.86 (m, 2H, C*H*$_2$-CH$_2$NH) and 1.74-1.55 (m, 1H, C*H*$_2$-CH); δ_C (400 MHz, MeOD) 170.9 (ester *C*=O), 159.0 (carbamate *C*=O), 138.4 (phenyl *C*), 129.6, 129.1, 128.8 (*C*H phenyl), 67.5 (*C*H$_2$-phenyl), 53.8 (O*C*H$_3$ and *C*H), 41.0 (*C*H$_2$-NH), 28.8 (*C*H$_2$-CH) and 26.6 (*C*H$_2$-CH$_2$NH).

3.42, 2-Methylpropanoylamino-(L)-Orn-(Cbz)-OMe

Amine **3.41** (2.49 g, 7.8 mmol) was dissolved in dichloromethane (50 mL), H_2O (20 mL) and cooled to 0 °C. Triethlyamine (3.2 mL, 23.4 mmol) and isobutyryl chloride (0.83 mL, 7.8 mmol) were added and the reaction stirred over night. The dichloromethane was removed *in vacuo* and the aqueous layer was extracted with

ethyl acetate. The organic layer was then washed with pH 2 buffer (3 × 15 mL), dried over Na_2SO_3 and reduced *in vacuo* to give compound **3.42** as a white solid (2.28 g, 83 %); δ_H (400 MHz, $CDCl_3$) 7.37-7.28 (m, 5H, phenyl), 6.28 (d, 1H, *J* 8, N*H*-CH), 5.15 (t, 1H, *J* 5.5, N*H*-CH_2), 5.09 (s, 2H, C*H*$_2$-phenyl), 4.60 (dt, 1H, *J* 7.5, 5.5, C*H*-NH), 3.73 (s, 3H, OC*H*$_3$), 3.59 (q, 2H, *J* 6.5, C*H*$_2$-NH), 2.42 (sextet, 1H, *J* 7, C*H*-$C(CH_3)_2$), 1.93-1.83 (m, 1H, C*H*$_2$-CH), 1.73-1.63 (m, 1H, C*H*$_2$-CH), 1.55 (quintet, 2H, *J* 7, C*H*$_2$-CH_2NH) 1.17 (d, 3H, *J* 7, (CH_3)$_2$) and 1.16 (d, 3H, *J* 7, (CH_3)$_2$); δ_C (100 MHz, $CDCl_3$) 177.0 (ester *C*=O), 173.0 (amide *C*=O), 156.6 (carbamate *C*=O), 136.6 (*C* phenyl), 128.5, 128.1 (phenyl *C*H), 66.6 (*C*H_2-phenyl), 52.4 (O*C*H_3), 51.6 (*C*H), 40.5 (*C*H_2-NH), 35.4 (*C*H$(CH_3)_2$), 29.7 (*C*H_2-CH), 26.0 (*C*H_2-CH_2NH) and 19.6, 19.4 (C(*C*H_3)$_2$; ESI *m/*z 100 %, 351.2 (MH^+), 12 %, 345.1 (MNa^+).

3.43, Propanoylamino-(L)-Orn-(Cbz)-OMe

Amine **3.41** (3.89 g, 12 mmol) was dissolved in dichloromethane (50 mL) and H_2O (20 mL) and cooled to 0 °C. Triethlyamine (5 mL, 36 mmol) and propionyl chloride (1 mL, 12 mmol) were added and the reaction stirred over night. The dichloromethane was removed *in vacuo* and the aqueous layer was extracted with ethyl acetate. The organic layer was then washed with pH 2 buffer (3 × 15 mL), dried over Na_2SO_3 and reduced *in vacuo* to give compound **3.43** as a white solid (2.58 g, 64 %); δ_H (400 MHz, $CDCl_3$) 7.37-7.29 (m, 5H, phenyl), 6.17 (d, 1H, *J* 7, N*H*-CH), 5.09 (s, 2H, C*H*$_2$-phenyl), 4.99 (br.s, 1H, N*H*-CH_2), 4.62 (q, 1H, *J* 7, C*H*-NH), 3.73 (s, 3H, OC*H*$_3$), 3.21 (q, 2H, *J* 6.5, C*H*$_2$-NH), 2.55 (q, 2H, *J* 7.5, C*H*$_2$-CH_3), 1.92-1.83 (m, 1H, C*H*$_2$-CH), 1.73-1.63 (m, 1H, C*H*$_2$-CH), 1.59-1.50 (m, 2H, C*H*$_2$-CH_2NH) and 1.15 (t, 3H, *J* 7, C*H*$_3$-CH_2); δ_C (100 MHz, $CDCl_3$) 173.7 (ester

C=O), 173.0 (amide *C*=O), 156.5 (carbamate *C*=O), 136.6 (phenyl *C*), 128.5, 128.1, (phenyl *C*H), 66.7 (*C*H_2-phenyl), 52.5 (O*C*H_3), 51.7 (*C*H), 40.5 (*C*H_2-NH), 29.8 (*C*H_2-CH), 29.5 (*C*H_2-CO), 26.0 (*C*H_2-CH_2NH) and 9.7 (*C*H_3-CH_2); ESI *m/*z 100 %, 337.1 (MH^+), 58 %, 359.1 (MNa^+).

3.44, Acetylamino-(L)-Orn-(Cbz)-OMe

Amine **3.41** (1.92 g, 6 mmol) was dissolved in dichloromethane (50 mL) and H_2O (30 mL) and cooled to 0 °C. Triethlyamine (2.5 mL, 18 mmol) and acetyl chloride (0.43 mL, 6 mmol) were added and the reaction stirred over night. The dichloromethane was removed *in vacuo* and the aqueous layer was extracted with ethyl acetate. The organic layer was then washed with pH 2 buffer (3 × 15 mL), dried over Na_2SO_3 and reduced *in vacuo* to give compound **3.44** as a white solid (1.76 g, 92 %); δ_H (400 MHz, $CDCl_3$) 7.36-7.26 (m, 5H, phenyl), 6.35 (d, 1H, *J* 8, N*H*-CH), 5.09 (s, 3H, C*H*$_2$-phenyl and N*H*-CH_2), 4.57 (q, 1H, *J* 7, C*H*-NH), 3.70 (s, 3H, OC*H*$_3$), 3.18 (q, 2H, *J* 7, C*H*$_2$-NH), 1.99 (s, 3H, C*H*$_3$), 1.88-1.75 (m, 1H, C*H*$_2$-CH), 1.70-1.60 (m, 1H, C*H*$_2$-CH) and 1.57-1.48 (m, 2H, C*H*$_2$-CH_2NH); δ_C (100 MHz, $CDCl_3$) 172.9, 170.1 (ester and amide *C*=O), 156.6 (carbamate *C*=O), 136.6 (phenyl *C*), 128.5, 128.13, 128.09 (phenyl *C*H), 66.7 (*C*H_2-phenyl), 52.5 (O*C*H_3), 51.8 (*C*H), 40.5 (*C*H_2-NH), 29.6 (*C*H_2-CH), 26.0 (*C*H_2-CH_2NH) and 23.1 (*C*H_3); ESI *m/*z 100 %, 323.1 (MH^+), 36 %, 345.1 (MNa^+).

3.45, 2-Methylpropanoylamino-(*S*)-tetrahydropyridin-2-one

Carbamate **3.42** (2.28 g, 6.51 mmol) was dissolved in MeOH (70 mL), palladium activated charcoal (0.7 g, catalytic) was added and the reaction stirred under hydrogen over night. The palladium was removed through filtration and the organic

solvents were removed *in vacuo* to give compound **3.45** as a white solid (1.06 g, 88 %) *mp* 129-130 °C; $[\alpha]^{24}_{D}$ (c = 0.24, $CHCl_3$) +112.85; υ_{max}/cm^{-1}: 3290, 3223 (N-H), 2933 (saturated C-H) and 1654, 1637 (C=O); *Anal.* ($C_9H_{16}N_2O_2$ requires: C; 58.67, H; 8.75, N; 15.21) found: C; 58.57, H; 8.74, N; 15.08; δ_H (400 MHz, MeOD) 4.60 (dd, 1H, *J* 10, 6, C*H*-NH), 3.536-3.34 (m, 2H, CH_2-NH obscured by MeOD), 2.55 (sextet, 1H, *J* 7, C*H*-C$(CH_3)_2$), 2.20-2.13 (m, 1H, CH_2-CH), 2.03-1.90 (m, 2H, CH_2-CH_2-NH) 1.84 (qd, 1H, *J* 11, 4, CH_2-CH) 1.21 (d, 3H, *J* 7, (CH_3)$_2$) and 1.20 (d, 3H, *J* 7, (CH_3)$_2$; δ_C (100 MHz, MeOD) 179.9, 173.1 (*C*=O), 50.7 (*C*H), 42.9 (lactam *C*H_2-NH), 36.2 (*C*H$(CH_3)_2$), 28.9 (lactam *C*H_2-CH), 22.4 (lactam *C*H_2-CH_2NH) and 20.0, 19.8 (C(*C*H_3)$_2$; ESI *m/*z 100 %, 391.0 (M_2Na^+), 78 %, 207.1 (MNa^+); HR ESI *m/*z ($C_9H_{16}N_2O_2Na$ requires 207.1104) found 207.1104.

3.46, Propanoylamino-(*S*)- tetrahydropyridin-2-one

Carbamate **3.43** (2.28 g, 6.80 mmol) was dissolved in MeOH (70 mL), palladium activated charcoal (0.7 g, catalytic) was added and the reaction stirred under hydrogen over night. The palladium was removed through filtration and the organic solvents were removed *in vacuo* to give compound **3.46** as a white solid (0.9 g, 78 %) *mp* 119-120 °C; $[\alpha]^{24}_{D}$ (c = 0.242, $CHCl_3$) +128.44; υ_{max}/cm^{-1}: 3292 (N-H), 2942 (saturated C-H) and 1620 (C=O); *Anal.* ($C_8H_{14}N_2O_2$ requires: C; 58.45, H; 8.26, N; 16.46) found: C; 56.19, H; 8.25, N; 16.13; δ_H (400 MHz, MeOD) 4.35 (dd, 1H, *J* 11, 6, C*H*), 3.36-3.32 (m, 2H, lactam CH_2-NH), 2.30 (q, 2H, *J* 8, CH_2-CH_3), 2.18-2.11 (m, 1H, lactam CH_2-CH), 2.10-1.87 (m, 2H, lactam CH_2-CH_2NH) 1.81 (qd, 1H, *J* 11.5, 4, lactam CH_2-CH) and 1.18 (t, 3H, *J* 7.5, CH_3-CH_2); δ_C (100 MHz, MeOD) 176.7, 173.1 (*C*=O amide), 50.9 (*C*H), 42.8 (lactam *C*H_2-NH), 30.1 (*C*H_2-CO), 28.9(lactam *C*H_2-CH), 22.4 (lactam *C*H_2-CH_2NH) and 10.3 (*C*H_3-CH_2); ESI *m/*z 100

%, 193.1 (MNa^+), 78 %, 363.0 (M_2Na^+); HR ESI *m/*z ($C_8H_{14}N_2O_2Na$ requires 193.0947) found 193.0958.

3.47, Acetylamino-(*S*)-tetrahydropyridin-2-one

Carbamate **3.44** (1.76 g, 5.46 mmol) was dissolved in MeOH (50 mL), palladium activated charcoal (0.5 g, catalytic) was added and the reaction stirred under hydrogen over night. The palladium was removed through filtration and the organic solvents were removed *in vacuo* to give compound **3.47** as a white solid (0.49 g, 58 %) *mp* 159-160 °C; $[\alpha]^{25}_D$ (c = 0.24, $CHCl_3$) +132.71; υ_{max}/cm^{-1}: 3290, 3219 (N-H), 2933 (saturated C-H) and 1627 (C=O); *Anal.* ($C_7H_{12}N_2O_2$ requires: C; 53.83, H; 7.74, N; 17.94) found: C; 53.58, H; 7.69, N; 17.48; δ_H (400 MHz, $CDCl_3$) 6.56 (br.s, 1H, N*H*-CH), 6.19 (br.s, 1H, N*H*-CH_2), 4.27 (dt, 1H, *J* 11.5, 6, C*H*), 3.34-3.30 (m, 2H, lactam C*H*$_2$-NH), 2.52 (ddt, 1H, *J* 13, 5.5, 4.5, equatorial C*H*$_2$-CH), 2.00 (s, 3H, C*H*$_3$-CO), 1.95-1.88 (m, 2H, lactam C*H*$_2$-CH_2NH) and 1.51 (tt, 1H, *J* 12, 8.5, lactam axial C*H*$_2$-CH); δ_C (100 MHz, $CDCl_3$) 171.9, 170.5 (*C*=O), 50.6 (*C*H), 41.7 (*C*H_2-NH), 27.2 (*C*H_2-CH), 22.3 (C*H*$_3$-CO) and 21.0 (*C*H_2-CH_2NH); ESI *m/*z 100 %, 179.1 (MNa^+), 62 %, 335.0 (MNa^+); HR ESI *m/*z ($C_7H_{12}N_2O_2Na$ requires 179.0791) found 179.0791. NMR Data is consistent with previously reported data for this compound.[242]

6.3.3 5-Membered Exupéry Compounds

3.48, *Nα*-Boc-(L)-2,4-diaminobutyric acid

N-Boc-(L)-Glutamine (2.47 g, 10.0 mmol) was dissolved in THF (35 mL) and H_2O (25 mL) and was cooled to 0 °C. Iodobenzene diacetate (3.59 g, 11.2 mmol) was added and the reaction stirred for 6 hours. The THF was removed *in vacuo* and the aqueous layer was extracted with diethyl ether. The aqueous layer was then reduced

in vacuo to give compound **3.48** as a light yellow solid (1.40 g, 64 %); δ_H (400 MHz, *d*^{6}DMSO) 6.18 (br.s, 1H, N*H*-COO), 3.57-3.52 (m, 1H, C*H*), 2.93-2.81 (m, 2H, C*H*$_2$-NH$_2$), 1.90-1.85 (m, 1H, C*H*$_2$-CH), 1.77-1.66 (m, 1H, C*H*$_2$-CH), and 1.40 (s, 9H, C(*C*H$_3$)$_3$); ESI *m/*z 100 %, 163.2 (MH^+-C_4H_8), 93 %, 219.2 (MH^+) and 7.5 % (MNa^+). Data is consistent with that previously reported.[206]

3.49, *N*α-Boc-(D)-2,4-diaminobutyric acid

N-Boc-(D)-Glutamine (1.76 g, 7.15 mmol) was dissolved in tetrahdrofuran (50 mL) and H_2O (20 mL) and was cooled to 0 °C. Iodobenzene diacetate (2.30 g, 7.15 mmol) was added and the reaction stirred over night. The THF was removed *in vacuo* and the aqueous layer was extracted with diethyl ether. The aqueous layer was then reduced *in vacuo* to give compound **3.49** as a light yellow solid (1.53 g, 98 %).

3.50, *N*α-Boc-*N*γ-Cbz-(L)-2,4-diaminobutyric acid

Amine **3.48** (7.64 g, 35.0 mmol) was dissolved in THF (80 mL) and H_2O (40 mL) and cooled to 0 °C. KOH (2.0 g, 36 mmol), K_2CO_3 (9.66 g, 70.0 mmol) and benzyl chloroformate (5.0 mL, 35 mmol) were added and the reaction was stirred over night. The THF was removed *in vacuo* and the aqueous layer was extracted with diethyl ether. The aqueous layer was then acidified to pH 4 with citric acid and extracted with ethyl acetate. The organic layer was then washed with NaCl (aq), dried over Na_2SO_3 and reduced *in vacuo* to give compound **3.50** as a colourless oil (10 g, 80 %); δ_H (400 MHz, *d*^{6}DMSO) 7.38-7.29 (m, 6H, phenyl and N*H*-Cbz), 7.09 (d, 1H, *J* 8, N*H*-CH), 5.00 (s, 2H, C*H*$_2$-phenyl), 3.91 (m, 1H, C*H*), 3.09-3.01 (m, 2H, C*H*$_2$-NH), 1.84 (m, 1H, C*H*$_2$-CH), 1.67 (m, 1H, C*H*$_2$-CH) and 1.36 (C(C*H*$_3$)$_3$; δ_C (100 MHz, *d*^{6}DMSO) 174.0 (acid *C*=O), 156.0, 155.6 (carbamate *C*=O), 137.2 (*C*

phenyl), 128.3, 127.7 (phenyl <u>C</u>H), 78.0 (<u>C</u>$(CH_3)_3$), 65.2 (<u>C</u>H_2-phenyl), 51.2 (<u>C</u>H), 37.5 (<u>C</u>H_2-NH), 30.8 (<u>C</u>H_2-CH) and 28.2 (C(<u>C</u>$H_3)_3$); ESI *m/*z 100 %, 375.1 (MNa^+), 70 %, 253.2 (MH^+-$C_5H_8O_2$) and 12 %, 297.1 (MH^+-C_6H_8). 1H data is available for this compound however run in another solvent.[206]

3.51, *N*α-Boc-*N*γ-Cbz-(D)-2,4-diaminobutyric acid

Amine **3.49** (1.56 g, 7.14 mmol) was dissolved in THF (50 mL) and H_2O (30 mL) and cooled to 0 °C. KOH (0.44 g, 7.86 mmol), K_2CO_3 (2.22 g, 16.01 mmol) and benzyl chloroformate (1.14 mL, 8 mmol) were added and the reaction was stirred over night. The THF was removed *in vacuo* and the aqueous layer was extracted with diethyl ether. The aqueous layer was then acidified to pH 4 with citric acid and extracted with ethyl acetate. The organic layer was then washed with NaCl (aq), dried over Na_2SO_3 and reduced *in vacuo* to give compound **3.51** as a colourless oil (1.46 g, 61 %); δ_H (400 MHz, $CDCl_3$) 7.37-7.29 (m, 5H, phenyl), 6.38 & 5.70 (br.t, 1H, N<u>*H*</u>-CH_2 rotamers), 5.39 & 5.44 (br.d, 1H, N<u>*H*</u>-CH rotamers), 5.12 (d, 1H, C<u>*H*</u>$_2$-phenyl), 5.05 (d, 1H, C<u>*H*</u>$_2$-phenyl), 4.40-4.33 (m, 1H, C<u>*H*</u>), 3.48, 3.31, 3.18 & 3.10 (m, 2H, C<u>*H*</u>$_2$-NH rotamers), 1.92 & 1.78 (m, 2H, C<u>*H*</u>$_2$-CH) and 1.42 (C(C<u>*H*</u>$_3)_3$; ESI *m/*z 100 %, 375.1 (MH^+-$C_5H_8O_2$) and 63 %, 253.1 (MNa^+).

3.52, *N*γ-Cbz-(L)-2,4-Diaminobutyric acid-OMe.HCl

Acetyl chloride (4 mL, 56 mmol) was dissolved in MeOH (70 mL) and cooled to 0 °C. Carbamate **3.50** (9.87 g, 28 mmol) was added and the reaction stirred on ice over night then reduced *in vacuo* to give compound **3.52** as a light brown oil (6 g, 71 %); δ_H (400 MHz, $CDCl_3$) 8.53 (br.s, 3H, N<u>*H*</u>), 7.29-7.25 (m, 5H, phenyl), 5.04 (d, 1H, *J*

12, C$\underline{H}_2$-phenyl), 5.00 (d, 1H, *J* 12, C$\underline{H}_2$-phenyl), 4.25-4.00 (m, 1H, C$\underline{H}$), 3.65 (s, 3H, OC$\underline{H}_3$), 3.24-3.31 (m, 2H, C$\underline{H}_2$-NH) and 2.29-2.20 (m, 2H, C$\underline{H}_2$-CH).

3.53, *N*γ-Cbz-(D)-2,4-Diaminobutyric acid-OMe.HCl

Acetyl chloride (1.2 mL, 17 mmol) was dissolved in MeOH (60 mL) and cooled to 0 °C, compound **3.51** (3 g, 8.51 mmol) was added and the reaction stirred over night then reduced *in vacuo* to give compound **3.53** as a light brown oil (2.42 g, 94 %).

3.54, Adamantante acetyl chloride

Adamantane carboxylic acid (1.47 g, 8.16 mmol) was dissolved in dichloromethane and cooled to 0 °C. Oxalyl chloride (0.71 mL, 8.16 mmol) and DMF (1 drop) were added and the reaction was stirred for 4 hours. The organic solvents were removed *in vacuo* to give compound **352** which was not isolated and reacted on.

3.55, Adamantane acetyl-*N*γ-Cbz-(L)-2,4-Diaminobutyric acid-OMe

Amine **3.52** (1.51 g, 5 mmol) was dissolved in THF (35 mL) and H_2O (20 mL) and cooled to 0 °C. Triethylamine (2.1 mmol, 15 mmol) and compound **3.54** (8 mmol) were added and stirred over night. The reaction was reduced *in vacuo*, dissolved in ethyl acetate, washed with pH 2 buffer (3 × 15 mL), the product was purified by silica column chromatography (petroleum ether:ethyl acetate 50:50) to give compound **3.55** as a colourless oil (0.96 g, 45 %); δ_H (400 MHz, $CDCl_3$) 7.37-7.27 (m, 5H, phenyl), 6.54 (d, 1H, *J* 8, N$\underline{H}$-CH), 5.86 (br.t, 1H, *J* 6, N$\underline{H}$-CH_2), 5.13 (d, 1H, *J* 12, C$\underline{H}_2$-phenyl), 5.08 (d, 1H, *J* 12, C$\underline{H}_2$-phenyl), 4.67 (td, 1H, *J* 8, 4, C$\underline{H}$), 3.72 (s, 3H, OC$\underline{H}_3$), 3.54-3.44 (m, 1H, C$\underline{H}_2$-NH), 2.99-2.91 (m, 1H, C$\underline{H}_2$-NH), 2.15-2.07 (m, 2H, C$\underline{H}_2$-CH), 2.07-2.03 (br.s, 3H, adamantane C$\underline{H}$-CH_2), 1.89 (br.s, 6H, adamantane C$\underline{H}_2$-CCO) and 1.73 (q, 6H, *J* 11, adamantane C$\underline{H}_2$-CH); δ_C (100 MHz,

$CDCl_3$) 178.5 (ester *C*=O), 173.1 (amide *C*=O), 156.6 (carbamate *C*=O), 136.6 (phenyl *C*), 128.4, 128.0 (phenyl *C*H), 66.5 (*C*H_2-phenyl), 52.5 (O*C*H_3), 49.3 (*C*H), 40.7 (adamantane *C*-CO), 39.1 (adamantane *C*H-CCO), 36.9 (*C*H_2-NH), 36.4 (adamantane *C*H_2-CH), 33.12 (*C*H_2-CH) and 28.0 (adamantane *C*H_2-CH_2); ESI *m/*z 100 %, 451.2 (MNa^+), 29 %, 429.2 (MH^+).

3.56, *tert*-Butanoyl-*N*γ-Cbz-(L)-2,4-Diaminobutyric acid-OMe

Amine **3.52** (5.71 g, 18.7 mmol) was dissolved in dichloromethane (80 mL) and cooled to 0 °C. Triethylamine (7.9 mL, 56.4 mmol) and trimethyl acetyl chloride (2.3 mL, 2.3 mmol) were added and stirred over night. The reaction was reduced *in vacuo* and dissolved in ethyl acetate, washed with pH 2 buffer (3 × 15 mL) reduced *in vacuo.* The product was then purified by silica column chromatography (petroleum ether:ethyl acetate 50:50) to give compound **3.54**6as a colourless oil (3.15 g, 48 %); δ_H (400 MHz, $CDCl_3$) 7.37-7.29 (m, 5H, phenyl), 6.61 (d, 1H, *J* 8, N*H*-CH), 5.76 (br.t, 1H, *J* 5, N*H*-CH_2), 5.12 (d, 1H, *J* 12, C*H*$_2$-phenyl), 5.08 (d, 1H, *J* 10, C*H*$_2$-phenyl), 4.66 (dt, 1H, *J* 8, 4, C*H*), 3.72 (s, 3H, OC*H*$_3$), 3.54-3.45 (m, 1H, C*H*$_2$-NH), 3.00-2.92 (m, 1H, C*H*$_2$-NH), 2.17-2.06 (m, 1H, C*H*$_2$-CH), 1.80-1.72 (m, 1H, C*H*$_2$-CH) and 1.23 (s, 9H, C(C*H*$_3$)$_3$); δ_C (100 MHz, $CDCl_3$) 179.2, 173.1 (*C*=O ester and amide), 156.7 (carbamate *C*=O), 136.6 (phenyl *C*), 128.4, 128.0 (phenyl *C*H), 66.6 (*C*H_2-phenyl), 52.5 (O*C*H_3), 49.6 (*C*H), 38.8 (*C*$(CH_3)_3$), 36.9 (*C*H_2-NH), 33.0 (*C*H_2-CH) and 27.4 (C(*C*H_3)$_3$); ESI *m/*z 100 %, 351.2 (MH^+) and 48 %, 373.2 (MNa^+).

3.57, *tert*-Butanoyl-*N*γ-Cbz-(D)-2,4-diaminobutyric acid-OMe

Amine **3.53** (2.42 g, 8 mmol) was dissolved in dichloromethane (50 mL) and cooled to 0 °C. Triethylamine (3.4 mL, 24 mmol) and trimethyl acetyl chloride (0.99 mL, 8 mmol) were added and stirred over night. The reaction was reduced *in vacuo* and dissolved in ethyl acetate, washed with pH 2 buffer (3 × 15 mL) reduced *in vacuo.* The product was then purified by silica column chromatography (petroleum ether:ethyl acetate 50:50) to give compound **3.57** as a colourless oil (0.51 g, 18 %); δ_H (400 MHz, $CDCl_3$) 7.38-7.28 (m, 5H, phenyl), 6.71 (d, 1H, *J* 8, N*H*-CH), 5.90 (br.t, 1H, *J* 6, N*H*-CH_2), 5.12 (d, 1H, *J* 12.5, C*H*$_2$-phenyl), 5.08 (d, 1H, *J* 12.5, C*H*$_2$-phenyl), 4.66 (ddd, 1H, *J* 16, 8, 5, C*H*), 3.70 (s, 3H, OC*H*$_3$), 3.53-3.43 (m, 1H, C*H*$_2$-NH), 3.04-2.95 (m, 1H, C*H*$_2$-NH), 2.15-2.06 (m, 1H, C*H*$_2$-CH), 1.87-1.78 (m, 1H, C*H*$_2$-CH) and 1.25 (s, 9H, C(C*H*$_3$)$_3$); δ_C (100 MHz, $CDCl_3$) 179.0 (ester *C*=O), 173.0 (amide *C*=O), 156.7 (carbamate *C*=O), 136.6 (phenyl *C*), 128.6, 128.1, (phenyl *C*H), 66.6 (*C*H_2-phenyl), 53.0 (O*C*H_3), 49.5 (*C*H), 38.8 (*C*$(CH_3)_3$), 36.9 (*C*H_2-NH), 32.5 (*C*H_2-CH) and 27.3 (C(*C*H_3)$_3$); ESI *m/*z 100 %, 351.2 (MH^+) and 12 %, 373.2 (MNa^+).

3.58, (*S*)-3-(Adamantanecarbonylamino)-pyrrolidin-2-one

Carbamate **3.55** (0.96 g, 2.24 mmol) was dissolved in MeOH (20 mL), palladium activated charcoal (0.18 g, catalytic) was added and the reaction stirred under hydrogen for 48 hours. The organic solvents were removed *in vacuo*, the product purified by recrystallisation from ethyl acetate to give compound **3.58** as a white solid (0.36 g, 61 %) *mp* 80-90 °C decomposed; $[\alpha]^{20}_D$ (c = 0.44, MeOH) - 26.04; υ_{max}/cm^{-1}: 3282 (N-H), 2901 (saturated C-H) and 1635 (C=O); δ_H (400 MHz, MeOD) 4.36 (t, 1H, *J* 10, C*H*), 3.34-3.22 (m, 2H, C*H*$_2$-NH), 2.46 (dddd, 1H, *J* C*H*$_2$-

CH), 2.00-1.90 (m, 4H, C$\underline{H}_2$-CH, adamantane C$\underline{H}$), 1.83 (d, 6H, *J* 3, adamantane C$\underline{H}_2$-CCO and 1.70 (q, 6H, *J* 10.5, adamantane C$\underline{H}_2$-CHCH$_2$); δ_C (100 MHz, MeOD) 181.0 (*C*=O lactam), 177.8 (*C*=O amide), 51.7 (*C*H), 41.9 (*C*H$_2$-NH), 40.1 (adamantane *C*H$_2$-CCO), 37.7 (adamantane *C*H$_2$-CHCH$_2$), 29.8 (adamantane *C*H) and 29.1 (*C*H$_2$-CH); ESI *m/*z 100 %, 547.1 (M_2Na^+), 56 %, 285.2 (MNa^+) and 11 %, 263.2 (MH^+); HR ESI *m/*z ($C_{15}H_{22}N_2O_2Na^+$ requires 285.1573) found 285.1578.

3.59, (*S*)-3-(2', 2'-Dimethylpropanoylamino)-pyrrolidin-2-one

Carbamate **3.56** was dissolved in MeOH, palladium activated charcoal was added and the reaction stirred under hydrogen for 24 hours. The charcoal was removed through filtration and the MeOH was removed *in vacuo*, the product was purified by recrystallisation was ethyl acetate to give compound **3.59** as a white solid (0.24 g, 33 %); *mp* 183-184 °C; $[\alpha]^{20}_D$ (c = 0.458, MeOH) - 37.15; υ_{max}/cm^{-1}: 3340, 3198 (N-H), 2961 (saturated C-H) and 1704, 1641 (C=O); δ_H (400 MHz, MeOD) 4.47 (dd, 1H, *J* 10, 9, C$\underline{H}$), 3.45-3.38 (m, 2H, C$\underline{H}_2$-NH), 2.48 (dddd, 1H, *J* 12.5, 9, 7, 1.5, C$\underline{H}_2$-CH), 2.08 (dq, 1H, *J* 12.5, 10, C$\underline{H}_2$-CH) and 1.24 (s, 9H, C(C$\underline{H}_3$)$_3$); δ_C (100 MHz, MeOD) 181.6 (lactam C=O), 177.8 (C=O), 51.9 (*C*H), 40.1 (*C*(CH$_3$)$_3$, 40.0 (*C*H$_2$-NH), 29.0 (*C*H$_2$-CH) and 27.8 (C(*C*H$_3$)$_3$); δ_H (400 MHz, $CDCl_3$) 6.72 (br.s, 1H, N$\underline{H}$-CH$_2$), 6.32 (br.d, 1H, *J* 4, N$\underline{H}$-CH), 4.25 (ddd, 1H, *J* 11, 8.5, 5, CH), 3.41-3.32 (m, 2H, C$\underline{H}_2$-NH), 2.48 (dddd, 1H, *J* 13, 9, 5, 3, C$\underline{H}_2$-CH), 2.08 (dq, 1H, *J* 12.5, 9.5, C$\underline{H}_2$-CH) and 1.19 (s, 9H, C(C$\underline{H}_3$)$_3$); δ_C (100 MHz, $CDCl_3$) 179.3 (*C*=O lactam), 176.1 (*C*=O amide), 51.0 (CH), 39.4 (*C*H$_2$-NH), 38.7 (*C*(CH$_3$)$_3$, 30.2 (*C*H$_2$-CH) and 27.5 (C(*C*H$_3$)$_3$); ESI *m/*z 100 %, 391.0 (M_2Na^+) and 22 %, 207.1 (MNa^+); HR ESI *m/*z ($C_9H_{16}N_2O_2H^+$ requires 185.1285) found 185.1279.

3.60, (*S*)-3-Amino-pyrrolidin-2-one

Acetyl chloride (2.0 mL, 28 mmol) was dissolved in MeOH (35 mL), L-2,4 diaminobutyric acid (1.25 g, 6.54 mmol) was added and the reaction stirred under reflux conditions for 3 hours. The reaction was cooled to under 0°C brought to pH 8 with NaOMe then stirred at room temperature for 48 hours. The reaction was deemed to be 50 % complete by NMR and the product compound **3.60** was used in the next step without purification.

3.61, Acetylamino-(*S*)-pyrrolidin-2-one

Lactam **3.60** (3.27 mmol) was dissolved in H_2O (5 mL), dichloromethane (15 mL) and cooled to 0 °C. Acetylchloride (0.70 mL, 9.8 mmol) in dichloromethane (5 mL) and triethylamine (4.10 mL, 29.4 mmol) were added and stirred over night. The reaction was reduced *in vacuo* purified by silica column chromatography (ethyl acetate:MeOH 100:0 to 80:20) to give compound **3.61** as a white solid (0.13 g, 14 %); *mp* 179-180 °C; $[\alpha]^{29}_D$ (c = 0.125, MeOH) -45.47; υ_{max}/cm^{-1}: 3271 (N-H) and 1693, 1635 (C=O); δ_H (400 MHz, MeOD) 4.53 (dd, 1H, *J* 10, 9, C*H*), 3.44-3.33 (m, 2H, lactam C*H*$_2$-NH), 2.51 (dddd, 1H, *J* 15, 8.5, 6, 2.5, lactam C*H*$_2$-CH), 2.07-1.96 (m, 1H, lactam C*H*$_2$-CH) and 2.03 (s, 3H, C*H*$_3$); δ_C (100 MHz, MeOD) 177.7 (lactam *C*=O), 173.5 (*C*=O), 51.7 (lactam *C*H), 40.0 (lactam *C*H$_2$-NH), 29.5 (lactam *C*H$_2$-CH) and 22.6 (*C*H$_3$); ESI *m/*z 100 %, 165.1 (MNa^+) and 98 %, 307.0 (M_2Na^+); HR ESI *m/*z ($C_6H_{10}N_2O_2Na^+$ requires 165.0634) found 165.0642. Data is consistent with previously reported data for this compound.[243]

3.62, Propanioylamino-(*S*)-pyrrolidin-2-one

Lactam **3.58** (3.27 mmol) was dissolved in H_2O (5 mL), dichloromethane (15 mL) and cooled on ice. Propionoyl chloride (0.80 mL, 9.8 mmol) in dichloromethane (5 mL) and triethylamine (4.10mL, 29.4 mmol) were added and stirred over night. The reaction was reduced *in vacuo* purified by silica column chromatography (ethyl acetate:MeOH 100:0 to 80:20) to give compound **3.62** as a white solid (0.072 g, 6 %); *mp* 151-152 °C; $[\alpha]^{31}_D$ (c = 0.135, MeOH) -76.05; υ_{max}/cm^{-1}: 3270 (N-H) and 1690, 1636 (C=O); δ_H (400 MHz, $CDCl_3$) 4.52 (dd, 1H, *J* 10.5, 9, C*H*), 3.39, 3.40 (2 × q, 2H, *J* 10, lactam C*H*$_2$-NH), 2.51 (dddd, 1H, *J* 15, 9, 6.5, 2.5, lactam C*H*$_2$-CH), 2.24 (qd, 2H, *J* 7, 2, C*H*$_2$-CH_3), 2.02 (ddt, 1H, *J* 12.5, 10.5, 9.5, lactam CH_2-CH) and 1.78 (t, 3H, *J* 7.5, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 177.8 (lactam C=O), 177.2 (C=O), 51.6 (lactam *C*H), 40.0 (lactam *C*H_2-NH), 30.0 (*C*H_2-CH_3), 29.5 (lactam *C*H_2-CH) and 10.2 (*C*H_3); ESI *m/*z 100 %, 335.0 (M_2Na^+) and 78 %, 179.1 (MNa^+); HR ESI *m/*z ($C_7H_{12}N_2O_2Na^+$ requires 179.0791) found 179.0793.

3.63, Isobutyrylamino-(*S*)-pyrrolidin-2-one

Lactam **3.60** (3.27 mmol) was dissolved in H_2O (5 mL) and dichloromethane (15 mL) and cooled to 0 °C. Isobutyryl chloride (1.40 mL, 13.4 mmol) in dichloromethane (5 mL) and triethylamine (5.60 mL, 40.2 mmol) were added and stirred over night. The reaction was reduced *in vacuo* purified by silica column chromatography (ethyl acetate:MeOH 100:0 to 80:20) to give compound **3.63** as a white solid (0.062 g, 6 %); *mp* 208-209 °C; $[\alpha]^{22}_D$ (c = 0.11, MeOH) -54.43; *Anal.* ($C_6H_{10}N_2O_2$ requires: C; 50.69, H; 7.69, N; 19.71) found: C; 50.58, H; 6.98, N; 19.51; υ_{max}/cm^{-1}: 3270 (N-H), 2972 (saturated C-H) and 1692 (C=O); δ_H (400 MHz, MeOD) 4.49 (dd, 1H, *J* 10, 9, lactam C*H*), 3.44-3.32 (m, 2H, lactam C*H*$_2$-NH), 2.57-

2.46 (m, 2H,lactam C$\underline{H}_2$-CH and C$\underline{H}(CH_3)_2$), 2.00 (ddt, 1H, *J* 12.5, 10, 9, equatorial C$\underline{H}_2$-CH), and 1.16, 1,18 (d, 3H, *J* 7, CH($\underline{C}H_3)_2$); δ_C (100 MHz, MeOD) 180.4, 177.7 ($\underline{C}$=O), 51.6 (lactam $\underline{C}$H), 40.0 (lactam $\underline{C}H_2$-NH), 36.2 ($\underline{C}$H-$(CH_3)_2$), 29.5 (lactam $\underline{C}H_2$-CH) and 19.8 (($\underline{C}H_3)_2$); ESI *m/*z 100 %, 363.0 (M_2Na^+) and 34 %, 193.1 (MNa^+); HR ESI *m/*z ($C_8H_{14}N_2O_2Na^+$ requires 193.0947) found 193.0948.

The other product isolation after column chromatography was a result of the (L)-2,4 diaminobutyric acid which did not cyclise in the synthesis of compound **3.58**. Consequently the isobutyryl chloride has added twice. δ_H (400 MHz, $CDCl_3$) 6.59 (br.t, 1H, *J* 5, N$\underline{H}$-CH_2), 6.40 (d, 1H, *J* 8, N$\underline{H}$-CH), 4.59 (ddd, 1H, *J* 10, 8.5, 4, C$\underline{H}$-NH), 3.73 (s, 3H, OC$\underline{H}_3$), 3.77-3.69 (m, 1H, C$\underline{H}_2$-NH), 2.75(ddt, 1H, *J* 14.5, 10, 4.5, C$\underline{H}_2$-NH), 2.09 (ddt, 1H, *J* 14, 10, 4.5, C$\underline{H}_2$-CH), 1.61 (ddt, 1H, *J* 14, 10.5, 4.5, C$\underline{H}_2$-CH) and 1.19-1.13 (m, 12H, CH($\underline{C}H_3)_2$); δ_C (100 MHz, $CDCl_3$) 177.9, 177.4, 173.1 ($\underline{C}$=O), 52.7 (O$\underline{C}H_3$), 49.3 ($\underline{C}$H), 40.0 (lactam $\underline{C}H_2$-NH), 36.6 ($\underline{C}$H-$(CH_3)_2$), 35.0 ($\underline{C}H_2$-NH), 33.2 ($\underline{C}H_2$-CH) and 19.7, 19.5 (($\underline{C}H_3)_2$); ESI *m/*z 100 %, 295.1 (MNa^+) and 95 %, 566.8 (M_2Na^+).

6.3.4 Reverse Compounds

3.64, (*S*)-*N*-1'-Adamantanyl pyrrolidin-2-one-5-carboxamide

(S)-(−)-2-Pyrrolidone-5-carboxylic acid (1.33 g, 10.3 mmol) was dissolved in dichloromethane (50 mL) and cooled to 0°C. HATU (3.86 g, 10.2 mmol) was added and the reaction stirred for 10 minutes. 1-Adamantamine.HCl (1.92 g, 10.2 mmol) and triethylamine (4.3 mL, 31 mmol) were added and the reaction was stirred over night. The dichloromethane was removed *in vacuo*, the product dissolved in ethyl acetate and washed with a pH 2 buffer (3 × 15 mL), 0.1M HCl (3 × 15 mL) and ½ saturated $NaHCO_3$ (3 × 15 mL). The organic layer was dried over Na_2SO_4 and

reduced *in vacuo*. The product was purified by recrystallation from dichloromethane to give compound **3.64** as a white solid (1.30 g, 48 %); *mp* 192-193 º C; $[\alpha]^{21}_{D}$ (c = 0.53, MeOH) 8.46; υ_{max}/cm^{-1}: 3503 (N-H), 2908 (saturated C-H) and 1653 (amide C=O); δ_H (500 MHz, $CDCl_3$) 7.23 (s, 1H, N*H*-CH), 5.84 (s, 1H, N*H*-C), 4.00 (dd, 1H, *J* 8, 5, C*H*-NHCO), 2.49-2.23 (m, 3H, lactam CH_2-CH and CH_2-CONH), 2.16-2.09 (m, 1H, lactam CH_2-CH), 2.05 (br.s, 3H, adamantane C*H*-CH_2), 1.96 (d, 6H, *J* 3, adamantane CH_2-CNH) and 1.64 (t, 6H, *J* 2.5, adamantane CH_2-CH); δ_C (100 MHz, $CDCl_3$) 179.6, 177.0 (*C*=O), 57.6 (C*H*-NHCO), 41.4 (adamantane *C*H_2-C), 36.3 (adamantane *C*H_2-CH), 29.6 (lactam CH_2-CONH), 29.4 (adamantane *C*H-CH_2) and 26.0 (lactam CH_2-CH_2-CONH); ESI *m/*z 100 %, 285.1 (MNa^+), 23 %, 547.1 (M_2Na^+); HR ESI *m/*z ($C_{15}H_{22}N_2O_2Na$ requires 285.1573) found 285.1573. Data consistent with reference.[244]

3.65, (*R*)-*N*-1'-Adamantanyl pyrrolidin-2-one-5-carboxamide

(*R*)-(+)-2-Pyrrolidone-5-carboxylic acid (1.55 g, 12.0 mmol) was dissolved in dichloromethane (90 mL) and cooled to 0°C. HATU (4.72 g, 12.4 mmol) was added and the reaction stirred for 4 hours. 1-Adamantamine.HCl (2.32 g, 12.4 mmol) and triethylamine (5.0 mL, 36 mmol) were added and the reaction was stirred over night. The dichloromethane was removed *in vacuo* and the product was dissolved in ethyl acetate and washed with a pH 2 buffer (3 × 15 mL), 0.1 M HCl (3 × 15 mL) and ½ saturated $NaHCO_3$ (3 × 15 mL). The organic layer was dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by washing with hot ethyl acetate to give compound **3.65** as a white solid (0.76 g, 24 %); *mp* 193-194 º C; $[\alpha]^{20}_{D}$ (c = 0.52, MeOH) -10.79; υ_{max}/cm^{-1}: 3305, 3242 (N-H), 2905 (saturated C-H) and 1673, 1639 (C=O); δ_H (400 MHz, $CDCl_3$) 4.01 (dd, 1H, *J* 9.5, 6.5, 1, C*H*-CONH), 2.50-2.41 (m,

2H, lactam C<u>*H*</u>$_2$-CONH), 2.39-2.24 (m, 1H, lactam C<u>*H*</u>$_2$-CH$_2$-CONH), 2.16-2.08 (m, 1H, lactam C<u>*H*</u>$_2$-CH$_2$-CONH), 2.05 (s, 3H, adamantane C<u>*H*</u>-CH$_2$), 1.97 (d, 6H, *J* 3, C<u>*H*</u>$_2$-C) and 1.65 (t, 6H, *J* 4, adamantane C<u>*H*</u>$_2$-CH); δ_C (100 MHz, $CDCl_3$) 179.6, 177.0 (<u>*C*</u>=O), 57.6 (C<u>*H*</u>-CONH), 41.4 (adamantane <u>*C*</u>H$_2$-C), 36.2 (adamantane <u>*C*</u>H$_2$-CH), 29.6 (lactam C<u>*H*</u>$_2$-CONH), 29.4 (adamantane <u>*C*</u>H-CH$_2$) and 26.0 (lactam <u>*C*</u>H$_2$-CH$_2$-CONH); *m/*z ESI 100 %, 263.2 (MH^+), 54 %, 525.2 (M_2H^+), 26 %, 547.2 (M_2Na^+) and 16 %, 285.2 (MNa^+); HR ESI ($C_{15}H_{22}N_2O_2Na$ requires 285.1573) found 285.1570.

3.66, (L)-Glu-(OMe)-OH.HCl

(L)-Glutamic acid (7.72 g, 52.5 mmol) was dissolved in MeOH (150 mL), chlorotrimethylsilane (13.3 mL, 105 mmol) was added dropwise. The clear solution was stirred for 10 minutes and then the solvent was removed *in vacuo* to give compound **3.66** as a white solid. ^{1}H NMR of the crude product showed the monomethylation as the major product (96 %), the crude product was used in the next step without further purification; δ_H (400 MHz, MeOD) 4.11 (t, 1H, *J* 7, C<u>*H*</u>), 3.74 (s, 3H, OC<u>*H*</u>$_3$), 2.68 (dt, 1H, *J* 16.5, 7, C<u>*H*</u>$_2$-COOCH$_3$), 2.62 (dt, 1H, *J* 16.5, 7, C<u>*H*</u>$_2$-COOCH$_3$), 2.29 (dq, 1H, *J* 14, 7, C<u>*H*</u>$_2$-CH) and 2.21 (dq, 1H, *J* 14, 7, C<u>*H*</u>$_2$-CH); δ_C (100 MHz, MeOD) 174.3, 171.4 (<u>*C*</u>=O), 53.3 (O-<u>*C*</u>H$_3$), 52.5 (<u>*C*</u>H), 30.5 (<u>*C*</u>H$_2$-COOMe) and 26.6 (<u>*C*</u>H$_2$-CH). Data is consistent with previously reported data for this compound. [206]

3.67, (D)-Glu-(OMe)-OH.HCl

(D)-Glutamic acid (7.36 g, 50.0 mmol) was dissolved in MeOH (150 mL), chlorotrimethylsilane (12.7 mL, 100 mmol) was added dropwise. The clear solution

was stirred for 10 minutes and then the solvent was removed *in vacuo* to give compound **3.67** as a white solid (9.6 g, 96 %). ^{1}H NMR of the crude product showed the monomethylation as the major product (96 %), the crude product was used in the next step without further purification; δ_H (400 MHz, MeOD) 8.52 (br.s, 1H, O*H*), 4.12 (t, 1H, *J* 7, C*H*), 3.74 (s, 3H, OCH_3), 2.69 (ddd, 1H, *J* 17, 8, 7, CH_2-COOCH_3), 2.64 (dt, 1H, *J* 17, 7.5, CH_2-COOCH_3), 2.29 (tt, 1H, *J* 14.5, 7, CH_2-CH) and 2.23 (tt, 1H, *J* 14.5, 7.5, CH_2-CH); δ_C (100 MHz, MeOD) 174.3, 171.4 (*C*=O ester), 53.3 (O-*C*H_3), 52.5 (*C*H), 30.5 (*C*H_2-COOMe) and 26.6 (*C*H_2-CH); ESI *mz* 100 %, 162.2 (MH^+-HCl).

3.68, *Nα*-Cbz-L-Glu-(OMe)-OH

Amine **3.66** (9.6 g, 48 mmol) was dissolved in dioxane (80 mL) and H_2O (30 mL) and the reaction cooled to 0 °C. Benzylchloroformate (8.24 mL, 57.6 mmol) and $NaHCO_3$ (10.1 g, 120 mmol) were added and stirred for 15 hours. The reaction was reduced *in vacuo*, dissolved in 10 % $NaHCO_3$ and washed with ether. The aqueous layer was then acidified to pH 4 using citric acid, extracted with ethyl acetate and wash with NaCl (3 × 20 mL). The organic layer was then dried over Na_2SO_4 and reduced *in vacuo* to give compound **3.68** as a colourless oil (3.87 g, 27 %); δ_H (400 MHz, MeOD) 7.40-7.29 (m, 5H, phenyl), 5.13 (d, 1H, *J* 12.5, CH_2-phenyl), 5.09 (d, 1H, *J* 12.5, CH_2-phenyl), 4.5-4.44 and 4.27-4.18 (1H, C*H* rotamers), 3.67 (s, 3H, OCH_3), 2.5-2.42 (m, 2H, CH_2-COOCH_3), 2.26-2.17 (m, 1H, CH_2-CH) and 2.01-1.90 (m, 1H, CH_2-CH); ESI *mz* 86 %, 318.0 (MNa^+).

3.69, *N*α-Cbz-(D)-Glu-(OMe)-OH

Amine **3.67** (9.6 g, 48 mmol) was dissolved in dioxane (70 mL) and H_2O (30 mL) and the reaction cooled to 0 °C. Benzylchloroformate (8.3 mL, 58 mmol) and $NaHCO_3$ (10.5 g, 125 mmol) were added and stirred for 15 hours. The reaction was reduced *in vacuo*, dissolved in 10 % $NaHCO_3$ and washed with ether. The aqueous layer was then acidified to pH 4 using citric acid, extracted with ethyl acetate and wash with NaCl (3 × 20 mL). The organic layer was then dried over Na_2SO_4 and reduced *in vacuo* to give compound **3.69** as a colourless oil (10.42 g, 73 %); δ_H (400 MHz, MeOD) 7.36-7.27 (m, 5H, phenyl), 5.73 (d, 1H, *J* 8, N*H*-CH), 5.11 (d, 1H, *J* 12, C*H*$_2$-phenyl), 5.07 (d, 1H, *J* 12, C*H*$_2$-phenyl), 4.6-4.53 and 4.45-4.3 (1H, C*H* rotamers), 3.65-3.60 (m, 3H, OC*H*$_3$), 2.48-2.37 (m, 2H, C*H*$_2$-COOCH$_3$), 2.27-2.16 (m, 1H, C*H*$_2$-CH) and 2.07-1.93 (m, 1H, C*H*$_2$-CH).

3.70, *N*α-Cbz-(L)-Glu-(OMe)-NHC(CH$_3$)$_3$

Acid **3.68** (8.0 g, 27 mmol) was dissolved in dichloromethane (50 mL) and cooled to 0° C. HATU (10.26 g, 26.98 mmol) was added and the mixture was stirred for 4 hours. Tertiary butylamine (2.8 mL, 27 mmol) and triethylamine (11.4 mL, 81 mmol) were added and the reaction was stirred over night. The dichloromethane was removed *in vacuo* and the product was dissolved in ethyl acetate and washed with a pH 2 buffer (3 × 20 mL), 0.1 M HCl (3 × 20 mL) and ½ saturated $NaHCO_3$ (3 × 20 mL) and purified by silica column chromatography (petroleum ether:ethyl acetate 50:50) to give compound **3.70** as a colourless oil 3.18 g (33 %); υ_{max}/cm^{-1}: 3336 (N-H), 2965 (saturated C-H), 1710 (ester C=O), 1657 (C=O), 1530 (aromatic); δ_H (400 MHz, $CDCl_3$) 7.36-7.29 (m, 5H, phenyl), 6.26 (s, 1H, N*H*-^{t}Bu), 5.88 (d, 1H, *J* N*H*-COO), 5.09 (s, 2H, C*H*$_2$-phenyl), 4.15 (m, 1H, C*H*), 3.66 (s, 3H, OC*H*$_3$), 2.48 (dt,

1H, *J* 16, 7.5, C*H*$_2$-COOCH$_3$), 2.38 (dt, 1H, *J* 16, 7, C*H*$_2$-COOCH$_3$), 2.12 (dq, 1H, *J* 21, 9.5, 6.5, C*H*$_2$-CH) and 2.12 (dq, 1H, *J* 7.5, 14, C*H*$_2$-CH); δ_C (100 MHz, $CDCl_3$) 173.8, 170.3 (*C*=O), 156.3 (carbamate *C*=O), 136.3 (phenyl *C*), 128.5 (*m*-phenyl *C*H), 128.1 (*p*-phenyl *C*H), 128.0 (*o*-phenyl *C*H), 66.9 (*C*H$_2$-phenyl), 54.4 (O-*C*H$_3$), 51.8 (*C*H), 51.4 (*C*(CH$_3$)$_3$), 30.1 (*C*H$_2$-COOMe), 28.6 (C(*C*H$_3$)$_3$) and 28.5 (*C*H$_2$-CH);ESI *m/*z 100 %, 373.2 (MNa^+).

3.71, *N*α-Cbz-(D)-Glu-(OMe)-NHC(CH$_3$)$_3$

Acid **3.69** (10.42 g, 35 mmol) was dissolved in dichloromethane (80 mL) and cooled to 0° C. HATU (12.8 g 33.7 mmol,) was added and the mixture was stirred for 4 hours. Tertiary butylamine (3.7 mL, 35 mmol) and triethylamine (14 mL, 105 mmol) were added and the reaction was stirred over night. The dichloromethane was removed *in vacuo* and the product was dissolved in ethyl acetate and washed with a pH 2 buffer (3 × 20 mL), 0.1 M HCl (3 × 20 mL) and ½ saturated $NaHCO_3$ (3 × 20 mL) and purified by silica column chromatography (petroleum ether:ethyl acetate 75:25) to give compound **3.71** as a colourless oil 3.98 g (32 %); υ_{max}/cm^{-1}: 3324 (N-H), 2967 (saturated C-H), 1732 (C=O ester), 1656 (C=O amide, carbamate) and 1529 (aromatic); δ_H (400 MHz, $CDCl_3$) 7.37-7.28 (m, 5H, phenyl), 6.38 (br.s, 1H, N*H*-^{t}Bu), 6.0 (d, 1H, *J* 8, N*H*-CH), 5.08 (s, 2H, C*H*$_2$-phenyl), 4.18 (m, 1H, C*H*), 3.66 (s, 3H, OC*H*$_3$), 2.47 (dt, 1H, *J* 16, 8, C*H*$_2$-COO), 2.38 (dt, 1H, *J* 16, 7, C*H*$_2$-COO), 2.10 (dq, 1H, *J* 14, 7, C*H*$_2$-CH), 1.94 (dq, 1H, *J* 14, 7, C*H*$_2$-CH) and 1.33 (s, 9H, C(C*H*$_3$)$_3$); δ_C (100 MHz, $CDCl_3$) 173.7, 170.4 (*C*=O), 156.3 (carbamate *C*=O), 136.3 (phenyl *C*), 128.5 (*m*-phenyl *C*H), 128.1 (*p*-phenyl *C*H), 127.9 (*o*-phenyl *C*H), 82.8 (*C*(CH$_3$)$_3$), 66.8 (*C*H$_2$-phenyl), 54.4 (O*C*H$_3$), 51.8 (*C*H), 30.1 (*C*H$_2$-COO), 28.6 (C(*C*H$_3$)$_3$) and 28.4 (*C*H$_2$-CH); ESI *m/*z 100 %, 373.2 (MNa^+), 19 %, 351.2 (MH^+).

3.72, (*S*)-*N*-1', 1'-Dimethylethyl pyrrolidin-2-one-5-carboxamide

Carbamate **3.70** (1.11 g, 3.17 mmol) was dissolved in MeOH (20 mL), palladium activated charcoal (0.2 g, catalytic) was added and the reaction stirred under hydrogen for 48 hours. The palladium was removed bu filtration, the organic solvents were removed *in vacuo* and the product was purified by recrystallisation from ethyl acetate and petroleum ether to give compound **3.72** as a white solid (0.2 g, 45 %); *mp* 151-152 °C; $[\alpha]^{21}_{D}$ (c = 0.5, MeOH) +16.43; υ_{max}/cm^{-1}: 3224 (N-H), 2968 (saturated C-H), 1683, 1647 (C=O); *Anal.* ($C_9H_{16}N_2O_2$ requires: C; 58.45, H; 8.77, N; 15.11) found: C; 58.67, H; 8.75, N; 15.21; δ_H (100 MHz, $CDCl_3$) 6.95 (s, 1H, N*H*), 5.93 (s, 1H, N*H*), 4.02 (ddd, 1H, 9, 5.5, 1, C*H*), 2.47 (tq, 1H, *J* 9, 6, C*H*$_2$-CH), 2.38 (ddd, 1H, *J* 18, 12, 6, C*H*$_2$-C=O), 2.30 (ddd, 1H, *J* 18, 12, 6, C*H*$_2$-C=O) and 1.34 (s, 9H, C(C*H*$_3$)$_3$); δ_C (100 MHz, MeOD) 178.8, 171.1 (*C*=O), 52.5 (*C*H), 51.6 (*C*(CH$_3$)$_3$), 29.5 (*C*H$_2$-CO) 28.7 (C(*C*H$_3$)$_3$) and 25.9 (*C*H$_2$-CH); ESI *m/*z 100 %, 391.1 (M_2Na^+), 16 %, 207.2 (MNa^+); HR ESI *m/*z ($C_9H_{16}N_2O_2Na^+$ requires 207.1104) found 207.1114; Data consistent with known compound. [245]

3.73, (*R*)-*N*-1', 1'-Dimethylethyl pyrrolidin-2-one-5-carboxamide

Carbamate **3.71** (3.98 g, 11.36 mmol) was dissolved in MeOH (40 mL), palladium activated charcoal (0.45 g) was added and the reaction stirred under hydrogen for 48 hours. The palladium was removed by filtration, the organic solvents were removed *in vacuo* and the product was purified by recrystallisation from ethyl acetate to give compound **3.73** as a white solid (0.3 g, 24 %) *mp* 139-142 °C (decomposed); $[\alpha]^{20}_{D}$ (c = 0.474, MeOH) -15.35; δ_H (400 MHz, MeOD) 7.60 (s, 1H, N*H*), 4.14 (dd, 1H, 8.5, 4.5, C*H*), 2.49-2.37 (m, 2H, C*H*$_2$-CH & C*H*$_2$-C=O), 2.35-2.27 (m, 1H, C*H*$_2$-C=O), 2.10-2.01 (m, 1H, C*H*$_2$-CH), 1.39 (s, 9H, C(C*H*$_3$)$_3$); δ_C (100 MHz, MeOD)

181.6, 174.1 (<u>C</u>=O), 58.5 (<u>C</u>H), 52.3 (<u>C</u>$(CH_3)_3$), 30.6 (<u>C</u>H_2-CO), 28.9 (C(<u>C</u>$H_3)_3$) and 26.8 (<u>C</u>H_2-CH); υ_{max}/cm^{-1}: 3302, 3222 (NH), 2971 (CH), 1685, 1648 (C=O); ESI *m/*z 100 %, 391.1 (M_2Na^+), 12 %, 207.2 (MNa^+); ESI *m/*z ($C_9H_{16}N_2O_2H^+$ requires 185.1285) found 185.1282.

3.74, *N*α-Cbz-(L)-Glu-(OMe)-NHCH$(CH_3)_2$

Acid **3.68** (1.41 g, 4.77 mmol) was dissolved in dichloromethane (20 mL) and cooled to 0° C. HATU (2.11 g 5.55 mmol) was added and the mixture was stirred for 4 hours. Isopropylamine (0.43 mL, 5 mmol) and triethylamine (2.1 mL, 15 mmol) were added and the reaction was stirred over night. The dichloromethane was removed *in vacuo* and the product was dissolved in ethyl acetate and washed with a pH 2 buffer (3 × 15 mL), 0.1 M HCl (3 × 15 mL) and ½ saturated $NaHCO_3$ (3 × 15 mL) and purified by silica column chromatography (petroleum ether:ethyl acetate 50:50) to give compound **3.74** as a colourless oil (0.80 g, 46 %); δ_H (400 MHz, $CDCl_3$) 7.35-7.29 (m, 5H, phenyl), 6.07 (br.s, 1H, N<u>H</u>-COO), 5.67 (d, 1H, *J* 7, N<u>H</u>-CH$(CH_3)_2$), 5.08 (s, 2H, C<u>H</u>$_2$-phenyl), 4.15 (br.q, 1H, *J* 8, C<u>H</u>-CH_2), 3.66 (s, 3H, OC<u>H</u>$_3$), 2.49 (dt, 1H, *J* 16.5, 7.5, C<u>H</u>$_2$-COO), 2.36 (dt, 1H, *J* 16.5, 7, C<u>H</u>$_2$-COO), 2.10 (dtd, 1H, *J* 14, 7, 5.5, C<u>H</u>$_2$-CH), 1.92 (quin., 1H, *J* 7.5, C<u>H</u>$_2$-CH), 1.13 (d, 3H, *J* 7, C(C<u>H</u>$_3)_2$) and 1.11 (d, 3H, *J* 7, C(C<u>H</u>$_3)_2$); δ_C (100 MHz, $CDCl_3$) 173.9, 170.1 (<u>C</u>=O), 163.3 (<u>C</u>=O carbamate), 138.0 (phenyl <u>C</u>), 128.6 (phenyl *m*-<u>C</u>H), 128.2 (phenyl *p*-<u>C</u>H), 128.1 (phenyl *o*-<u>C</u>H), 67.0 (<u>C</u>H_2-phenyl), 54.2 (O<u>C</u>H_3), 51.9 (<u>C</u>H), 41.6 <u>C</u>H$(CH_3)_2$, 30.2 (<u>C</u>H_2-COO), 28.4 (<u>C</u>H_2-CH) and 22.6 (CH(<u>C</u>$H_3)_2$); ESI *m/*z 100 %, 359.1 (MNa^+), 34 %, 336.0 (MH^+) and 20 % 694.5 (M_2Na^+).

3.75, (*S*)-*N*-1'-Methylethyl pyrrolidin-2-one-5-carboxamide

Carbamate **3.74** (0.8 g, 2.18 mmol) was dissolved in MeOH (30 mL), palladium activated charcoal (0.2 g, catalytic) was added and the reaction stirred under hydrogen for 48 hours. The palladium was removed by filtration, the organic solvents were removed *in vacuo* and the product was purified by recrystallisation from ethyl acetate and petroleum ether to give compound **3.75** as a white solid (0.265 g, 72 %) *mp* 134-135 °C; $[\alpha]^{31}_{D}$ (c = 0.474, MeOH) +4.42; ; υ_{max}/cm^{-1}: 3298 (N-H), 2969 (saturated C-H) and 1701, 1650 (C=O); δ_H (400 MHz, MeOD) 4.16 (dd, 1H, *J* 9.5, 5, C*H*-CH_2), 4.01 (septet, 1H, *J* 7, C*H*$(CH_3)_2$), 2.56-2.40 (m, 2H, C H_2-CH and CH_2-COO), 2.38-2.28 (m, 1H, CH_2-COO), 2.12-2.02 (m, 1H, CH_2-CH), 1.20 (d, 3H, *J* 7.5, (CH_3)$_2$) and 1.19 (d, 3H, *J* 7.5, C(CH_3)$_2$); δ_C (100 MHz, MeOD) 181.6, 173.9 (*C*=O), 58.3 (*C*H), 42.7 *C*H$(CH_3)_2$, 30.6 (*C*H_2-COO), 26.8 (*C*H_2-CH) and 22.4 (CH(*C*H_3)$_2$); ESI *m/*z 100 %, 363.0 (M_2Na^+) and 18 %, 193.1 (MNa^+); ESI *m/*z ($C_8H_{14}N_2O_2Na^+$ requires 193.0947) found 193.0950.

6.3.5 7-Membered Exupéry Compounds

3.76, Acetylamino-(*S*)-azepan-2-one

Lactam **2.65** (1.61 g, 9.82 mmol) was dissolved in dichloromethane (35 mL) and cooled on ice. Acetylchloride (0.70 mL, 10 mmol) in dichloromethane (5 mL) and triethylamine (2.8 mL, 20 mmol) were added and the reaction stirred over night. Diethyl ether (60 mL) was added and the precipitate removed by filtration, the filtrate was reduced *in vacuo* and the product purified by silica column chromatography (ethyl acetate:MeOH 100:0 to 80:20) to give compound **3.76** as a white solid (1.00 g, 60 %); *mp* 143-144 °C; $[\alpha]^{28}_{D}$ (c = 0.258, $CHCl_3$) +82.82; *Anal.* ($C_8H_{14}N_2O_2$ requires:

C; 56.45, H; 8.29, N; 16.46) found: C; 56.04, H; 8.25, N; 15.83; υ_{max}/cm^{-1}: 3283 (N-H), 2928 (saturated C-H) and 1650 (C=O); δ_H (400 MHz, $CDCl_3$) 6.89 (br.s, 1H, N*H*-CH), 6.35 (br.s, 1H, N*H*-CH_2), 4.51 (ddd, 1H, *J* 13, 6, 2, C*H*), 3.32-3.17 (m, 2H, lactam C*H*$_2$-NH), 2.06 (br.d, 1H, *J* 14.5, equatorial C*H*$_2$-CH), 2.00-1.94 (m, 1H, C*H*$_2$-CH_2NH), 1.99 (s, 3H, C*H*$_3$), 1.87-1.78 (m, 2H, lactam C*H*$_2$-CH_2CH) and 1.51-1.31 (m, 2H, lactam C*H*$_2$-CH and C*H*$_2$-CH_2NH); δ_C (100 MHz, $CDCl_3$) 175.7, 169.2 (*C*=O), 52.2 (lactam *C*H), 42.2 (lactam *C*H_2-NH), 31.7 (lactam *C*H_2-CH), 28.9 (lactam *C*H_2-CH_2CH), 27.9 (lactam *C*H_2-CH_2NH) and 23.3 (*C*H_3-CO); ESI *m/*z 100 %, 193.1 (MNa^+) and 89 %, 362.9 (M_2Na^+); HR ESI *m/*z ($C_8H_{14}N_2O_2Na^+$ requires 193.0947) found 193.0946. Data is consistent with previously reported data for this compound.[209]

3.77, Propanoyllamino-(*S*)-azepan-2-one

Lactam **2.65** (2.02 g, 12.3 mmol) was dissolved in dichloromethane (25 mL) and cooled on ice. Propanioylchloride (1.10 mL, 12.3 mmol) in dichloromethane (5 mL) and triethylamine (3.5 mL, 25 mmol) were added and the reaction stirred over night. Diethyl ether (60 mL) was added and the precipitate removed by filtration, the filtrate was reduced *in vacuo* and the product purified by silica column chromatography (ethyl acetate:MeOH 100:0 to 80:20) to give compound **3.77** as a white solid (1.47 g, 65 %); *mp* 135-136 °C; $[\alpha]^{27}_D$ (c = 0.232, $CHCl_3$) +60.34; υ_{max}/cm^{-1}: 3341, 3313 (N-H), 2938 (saturated C-H) and 1676 (C=O); δ_H (400 MHz, $CDCl_3$) 6.86 (br.s, 1H, N*H*-CH), 6.26 (br.s, 1H, N*H*-CH_2), 4.51 (ddd, 1H, *J* 11, 6, 1.5, C*H*), 3.32-3.18 (m, 2H, lactam C*H*$_2$-NH), 2.23 (q, 2H, C*H*$_2$-CO), 2.06 (br.d, 1H, *J* 14.5, equatorial lactam C*H*$_2$-CH), 2.00-1.94 (m, 1H, lactam C*H*$_2$-CH_2NH), 1.88-1.75 (m, 2H, lactam C*H*$_2$-CH_2CH), 1.50-1.34 (m, 2H, lactam C*H*$_2$-CH and C*H*$_2$-

CH_2NH) and 1.14 (t, 3H, *J* 7.5, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 175.8, 173.0 (*C*=O), 52.1 (lactam *C*H), 42.2 (lactam *C*H_2-NH), 31.7 (*C*H_2-CO), 29.7 (lactam *C*H_2-CH), 28.9 (lactam *C*H_2-CH_2CH), 28.0 (lactam *C*H_2-CH_2NH) and 9.73 (*C*H_3); ESI *m/*z 100 %, 207.1 (MNa^+) and 71 %, 390.9 (M_2Na^+); HR ESI *m/*z ($C_9H_{16}N_2O_2Na^+$ requires 207.1104) found 207.1105.

3.78, 2-Methylpropanoyllamino-(*S*)-azepan-2-one

Lactam **2.65** (1.64 g, 10.0 mmol) was dissolved in dichloromethane (35 mL) and cooled on ice. Isobutyrylchloride (1.1 mL, 10 mmol) in dichloromethane (5 mL) and triethylamine (2.8 mL, 20 mmol) were added and the reaction stirred over night. Diethyl ether (60 mL) was added and the precipitate removed by filtration, the filtrate was reduced *in vacuo* and the product purified by silica column chromatography (ethyl acetate:MeOH 100:0 to 80:20) to give compound **3.78** as a white solid (1.29 g, 65 %); *mp* 154-155 °C; $[\alpha]^{28}_D$ (c = 0.248, $CHCl_3$) +45.23; *Anal.* ($C_{10}H_{18}N_2O_2$ requires: C; 60.58, H; 9.15, N; 14.13) found: C; 60.44, H; 9.19, N; 14.00; υ_{max}/cm^{-1}: 3285 (N-H), 2927 (saturated C-H) and 1665, 1636 (C=O); δ_H (400 MHz, $CDCl_3$) 6.90 (br.d, 1H, *J* 5, N*H*-CH), 6.57 (br.s, 1H, N*H*-CH_2), 4.48 (ddd, 1H, *J* 11, 6, 2, C*H*), 3.30-3.16 (m, 2H, lactam C*H*$_2$-NH), 2.39 (sep., 1H, *J* 7, C*H*$(CH_3)_2$), 2.62 (br.d, 1H, *J* 14, equatorial C*H*$_2$-CH), 1.98-1.92 (m, 1H, lactam C*H*$_2$-CH_2NH), 1.85-1.92 (m, 2H, lactam C*H*$_2$-CH_2CH), 1.48-1.31 (m, 2H, lactam C*H*$_2$-CH and C*H*$_2$-CH_2NH) and 1.13, 1,11 (d, 3H, *J* 7, CH(*C*$H_3)_2$); δ_C (100 MHz, $CDCl_3$) 176.2, 176.0 (*C*=O), 51.9 (lactam *C*H), 42.1 (lactam *C*H_2-NH), 35.4 (*C*H-$(CH_3)_2$), 31.7 (lactam *C*H_2-CH), 28.9 (lactam *C*H_2-CH_2CH), 28.0 (lactam *C*H_2-CH_2NH) and 19.6, 19.4 ((*C*$H_3)_2$); ESI *m/*z 100 %, 419.0 (M_2Na^+) and 45 %, 221.1 (MNa^+); HR ESI *m/*z ($C_{10}H_{18}N_2O_2Na^+$ requires 221.1260) found 221.1261.

6.4 Chapter 4 Experimental

6.4.1 4-Carboxy Compounds

4.01, 4-Ethylbenzoyl chloride

4-Ethylbenzoic acid (1.55 g, 10.3 mmol) was dissolved in dichloromethane (25 mL), oxalyl chloride (0.88 mL, 10.3 mmol) and DMF (1 drop) were added and the reaction was stirred for 3 hours. The organic solvents were removed *in vacuo* to give compound **4.01** which was not isolated and used in the next stage.

4.02, 4-Butylbenzoyl chloride

4-Butylbenzoic acid (1.84 g, 10.3 mmol) was dissolved in dichloromethane (30 mL), oxalyl chloride (0.88 mL, 10.3 mmol) and DMF (1 drop) were added and the reaction was stirred for 3 hours. The organic solvents were removed *in vacuo* to give compound **4.02** which was not isolated and used in the next stage.

4.03, 4-*tert*-Butylbenzoyl chloride

4-*tert*-Butylbenzoic acid (2.04 g, 11.45 mmol) was dissolved in dichloromethane (40 mL), oxalyl chloride (0.98 mL, 11.45 mmol) and DMF (1 drop) were added and the reaction was stirred for 3 hours. The organic solvents were removed *in vacuo* to give compound **4.03** which was not isolated and used in the next stage.

4.05, 4-Octylbenzoyl chloride

4-Octylbenzoic acid (0.54 g, 2.22 mmol) was dissolved in dichloromethane (15 mL), oxalyl chloride (0.2 mL, 2.23 mmol) was added and DMF (1 drop) the mixture was

stirred for 3 hours. The organic solvents were removed *in vacuo* to give compound **4.05** which was not isolated and used in the next stage.

4.06, 4-Ethylbenzoyl-(*S*)-3-amino-azepan-2-one

Lactam **2.58** (1.65 g, 10 mmol) was dissolved in H_2O (20 mL) and cooled to 0°C. Acid chloride **4.01** (10.3 mmol) in dichloromethane (25 mL) and triethylamine (4.2 mL, 30 mmol) were added and stirred over night. H_2O (20 mL) was added and the reaction was extracted with dichloromethane (3 × 20 mL), the organic layer was washed with a pH 2 buffer (3 × 15 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by recrystallisation from chloroform and cold petroleum ether to give compound **4.06** as a white solid 0.94 g (36 %); *mp* 218-219 º C; $[\alpha]^{23}_D$ (c = 0.49, $CHCl_3$) +70.48; υ_{max}/cm^{-1}: 3200 (N-H), 2956 (saturated C-H), 1642 (C=O) and 1543 (aromatic); *Anal.* ($C_{15}H_{20}N_2O_2$ requires: C; 69.20, H; 7.74, N; 10.76) found: C; 69.13, H; 7.75, N; 10.71; δ_H (400 MHz, $CDCl_3$) 7.66 (d, 2H, *J* 8, C*H*-CO), 7.62 (d, 1H, *J* 5.5, N*H*-CH), 7.24 (d, 2H, *J* 8, C*H*-CEt), 6.55 (br.t, 1H, *J* 6, N*H*-CH_2), 4.70 (dd, 1H, *J* 11, 5.5, lactam C*H*), 3.37-3.32 (m, 2H, lactam C*H*$_2$-NH), 2.67 (q, 2H, *J* 7.5, C*H*$_2$-aryl), 2.21 (br.d, 1H, *J* 13, lactam equatorial C*H*$_2$-CH), 2.02 (dt, 1H, *J* 14, 4, lactam equatorial C*H*$_2$-CH_2CH), 1,95-1.82 (m, 2H, lactam equatorial C*H*$_2$-CH_2NH and axial C*H*$_2$-CH_2CH), 1.53 (br.q, 1H, *J* 12.5, lactam axial C*H*$_2$-CH), 1.40 (br.q, 1H, *J* 13, lactam axial C*H*$_2$-CH_2NH) and 1.22 (t, 3H, *J* 7.5, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 175.9 (lactam C=O), 166.2 (C=O), 148.2 (*C*-Et), 131.6 (*C*-CO), 128.0 (*C*H-CEt), 127.2 (*C*H-CO), 52.6 (*C*H-NH), 42.2 (lactam *C*H_2-NH), 28.9 (*C*H_2-aryl), 28.8 (lactam C*H*$_2$-CH), 28.0, 27.9 (lactam C*H*$_2$-CH_2-NH and C*H*$_2$-CH_2CH) and 15.4 (*C*H_3); ESI *m/*z 100 %, 542.9 (M_2Na^+), 70%, 283.1 (MNa^+) and 10 %, 261.2 (MH^+); HR ESI *m/*z ($C_{15}H_{20}N_2O_2Na$ requires 283.1417) found 283.1414.

4.07, 4-Ethylbenzoyl-(*R*)-3-amino-azepan-2-one

Lactam **2.66** (0.12 g, 0.47 mmol) was dissolved in H_2O (5 mL) and cooled to 0°C. Acid chloride **4.01** (1.60 mmol) in dichloromethane (10 mL) was added and triethylamine (2.1 mL, 1.5 mmol) and the reaction was stirred over night. H_2O (15 mL) was added and the reaction was extracted with dichloromethane (3 × 15 mL), the organic layer was washed with a pH 2 buffer (3 × 15 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 75:25:0 to 0:90:10 to give compound **4.07** as a white solid 0.027 g (22 %); $[\alpha]^{27}_D$ (c = 0.306, $CHCl_3$) -64.60; υ_{max}/cm^{-1}: 3200 (NH), 2925 (C-H), 1647 (amide C=O) and 1571 (aromatic); *mp* 217-218 º C; δ_H (400 MHz, $CDCl_3$) 7.76 (d, 2H, *J* 8.5, C*H*-CO), 7.60 (d, 1H, *J* 5.5, N*H*-CH), 7.26 (d, 2H, *J* 8.5, C*H*-CEt), 6.04 (br.s, 1H, N*H*-CH_2), 4.61 (ddd, 1H, *J* 11, 5.5, 2, lactam C*H*), 3.40-3.22 (m, 2H, lactam CH_2-NH), 2.68 (q, 2H, *J* 8, CH_2-aryl), 2.24 (br.d, 1H, *J* 13.5, lactam equatorial CH_2-CH), 2.08-2.01 (m, 1H, lactam equatorial CH_2-CH_2CH), 1.97-1.85 (m, 2H, lactam equatorial CH_2-CH_2NH and axial CH_2-CH_2CH), 1.55 (br.q, 1H, *J* 12.5, lactam axial CH_2-CH), 1.43 (br.q, 1H, *J* 13.5, lactam axial CH_2-CH_2NH) and 1.22 (t, 3H, *J* 7.5, CH_3); δ_C (100 MHz, $CDCl_3$) 175.9, 166.2 (*C*=O), 148.2 (*C*-Et), 131.6 (*C*-CO), 128.0 (*C*H-CEt), 127.2 (*C*H-CO), 52.6 (*C*H-NH), 42.3 (lactam *C*H_2-NH), 31.7 (lactam *C*H_2-CH), 29.0 (*C*H_2-aryl), 28.8, 28.0 (lactam *C*H_2-CH_2NH and *C*H_2-CH_2CH) and 15.4 (C6); ESI *m/*z 100 %, 283.1 (MNa^+) and 37 %, 542.8 (M_2Na^+); HR ESI *m/*z ($C_{15}H_{20}N_2O_2Na^+$ requires 283.1417) found 283.1413.

4.08, 4-Ethyl benzoyl-(*S*)-3-amino-tetrahydropyridin-2-one

Lactam **2.53** (20 mmol) was dissolved in H_2O (25 mL) and cooled to 0°C. Acid chloride **4.01** (16 mmol) in dichloromethane (30 mL) and triethylamine (6.7 mL, 48

mmol) were added and stirred over night. The reaction was extracted with dichloromethane (3 × 30 mL), the organic layer was washed with a pH 2 buffer (3 × 20 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 50:50:0 to 0:80:20) to give compound **4.08** as a white solid 1.46 g (37 %); *mp* 112-113 º C; $[\alpha]^{23}_D$ (c = 0.491, $CHCl_3$) +103.95; υ_{max}/cm^{-1}: 3334, 3245 (N-H), 2932 (saturated C-H), 1656, 1634 (C=O) and 1528 (aromatic); δ_H (400 MHz, $CDCl_3$) 7.71 (d, 2H, *J* 8.5, C*H*-CO), 7.55 (d, 1H, *J* 5.5, N*H*-CH), 7.19 (d, 2H, *J* 8.5, C*H*-CEt), 6.69 (br.s, 1H, N*H*-CH_2), 4.39 (dt, 1H, *J* 11, 5.5, C*H*-CH_2), 3.35-3.28 (m, 2H, lactam C*H*$_2$-NH), 2.67 (q, 2H, *J* 7.5, C*H*$_2$-aryl), 2.63-2.56 (m, 1H, lactam equatorial C*H*$_2$-CH), 1.94-1.87 (m, 2H, lactam C*H*$_2$-CH_2NH), 1.68 (tt, 1H, *J* 12.5, 8, lactam axial C*H*$_2$-CH), and 1.20 (t, 3H, *J* 7.5, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 172.2, 167.5 (*C*=O), 148.2 (*C*-Et), 131.5 (*C*-C=O), 127.9 (*C*H-CEt), 127.2 (*C*H-CO), 50.9 (*C*H-NH), 41.7 (C1), 28.8 (C4), 27.2 (C3), 21.1 (C2) and 15.3 (C5); ESI *m/*z 100 %, 514.9 (M_2Na^+) and 35%, 269.1 (MNa^+); HR ESI *m/*z ($C_{14}H_{18}N_2O_2Na$ requires 269.1260) found 269.1261.

4.09, 4-Ethyl benzoyl-(*R*)-3-amino-tetrahydropyridin-2-one

Lactam **2.54** (10 mmol) was dissolved in H_2O (25 mL) and cooled to 0°C. Acid chloride **4.01** (9 mmol) in dichloromethane (25 mL) and triethylamine (3.8 mL, 27 mmol) were added and stirred over night. The reaction was extracted with dichloromethane (3 × 30 mL), the organic layer was washed with a pH 2 buffer (3 × 20 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 50:50:0 to 0:80:20) to give compound **4.09** as a white solid 1.00 g (45 %); *mp* 109-110 º C; $[\alpha]^{28}_D$ (c = 0.51, $CHCl_3$) -95.54; υ_{max}/cm^{-1}: 3335, 3245 (N-H), 2962 (saturated C-H), 1666 (C=O) and

1545 (aromatic); δ_H (400 MHz, $CDCl_3$) 7.74 (d, 2H, *J* 8, C*H*-CO), 7.23 (d, 2H, *J* 8, C*H*-CEt), 7.18 (d, 1H, *J* 4.5, N*H*-CH), 6.06 (br.s, 1H, N*H*-CH_2), 4.42 (dt, 1H, *J* 12, 4.5, C*H*-CH_2), 3.39-3.35 (m, 2H, lactam C*H*$_2$-NH), 2.72 (ddt, 1H, *J* 13, 6, 4.5, lactam equatorial C*H*$_2$-CH), 2.67 (q, 2H, *J* 7.5, C*H*$_2$-aryl), 2.04-1.94 (m, 2H, lactam C*H*$_2$-CH_2NH), 1.60-1.56 (m, 1H, lactam axial C*H*$_2$-CH) and 1.22 (t, 3H, *J* 8, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 171.9, 167.6 (*C*=O), 148.3 (*C*-Et), 131.5 (*C*-CO), 128.0 (*C*H-CEt), 127.2 (*C*H-CO), 51.1 (*C*H-NH), 41.8 (lactam *C*H_2-NH), 28.8 (*C*H_2-aryl), 27.2 (lactam *C*H_2-CH), 21.1 (lactam *C*H_2-CH_2NH) and 15.3 (*C*H_3); ESI *m/*z 100 %, 514.9 (M_2Na^+) and 26 %, 269.1 (MNa^+); HR ESI *m/*z ($C_{14}H_{18}N_2O_2Na^+$ requires 269.1260) found 269.1258.

4.10, 4-Butylbenzoyl-(*S*)-3-amino-azepan-2-one

Lactam **2.65** (1.65 g, 10 mmol) was dissolved in H_2O (20 mL) and cooled to 0°C. Acid chloride **4.02** (8 mmol) in dichloromethane and triethylamine (4.2 mL, 30 mmol) were added and stirred over night. H_2O (20 mL) was added and the reaction was extracted with dichloromethane (3 × 20 mL), the organic layer was washed with a pH 2 buffer (3 × 15 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 75:25 to 0:100) to give compound **4.10** as a white solid 0.42 g (18 %); *mp* 183-184 º C; $[\alpha]^{25}_D$ (c = 0.515, $CHCl_3$) +64.88; υ_{max}/cm^{-1}: 3359, 3207 (N-H), 2951 (saturated C-H), 1671, 1650 (C=O) and 1543 (aromatic); *Anal.* ($C_{17}H_{24}N_2O_2$ requires: C; 70.80, H; 8.39, N; 9.70) found: C; 70.74, H; 8.37, N; 9.70; δ_H (400 MHz, $CDCl_3$) 7.73 (d, 2H, *J* 8, C*H*-CO), 7.63 (d, 1H, *J* 5.5, N*H*-CH), 7.21 (d, 2H, *J* 8, C*H*-C-^{n}Bu), 6.80 (br.t, 1H, *J* 6, N*H*-CH_2), 4.69 (dd, 1H, *J* 10.5, 5.5, C*H*), 3.35-3.20 (m, 2H, lactam C*H*$_2$-NH), 2.61 (t, 2H, *J* 7.5, C*H*$_2$-aryl), 2.19 (br.d, 1H, *J* 13.5, lactam equatorial

C<u>*H*</u>$_2$-CH), 2.00 (br.d, 1H, *J* 12.5, lactam equatorial C<u>*H*</u>$_2$-CH$_2$CH), 1.93-1.80 (m, 2H, lactam equatorial C<u>*H*</u>$_2$-CH$_2$NH and axial C<u>*H*</u>$_2$-CH$_2$CH), 1.57 (quintet, 2H, *J* 7.5, C<u>*H*</u>$_2$-CH$_2$-aryl), 1.55-1.46 (m, 1H, lactam axial C<u>*H*</u>$_2$-CH), 1.38 (br.q, 1H, *J* 13, lactam axial C<u>*H*</u>$_2$-CH$_2$CH), 1.31 (sextet, H2, *J* 7, C<u>*H*</u>$_2$-CH$_3$) and 0.89 (t, 3H, *J* 7.5, C<u>*H*</u>$_3$); δ_C (100 MHz, $CDCl_3$) 176.0, 166.3 (<u>*C*</u>=O), 146.7 (<u>*C*</u>-^{n}Bu), 131.6 (<u>*C*</u>-CO), 128.5 (<u>*C*</u>H-C^{n}Bu), 127.1 (<u>*C*</u>H-CO), 52.5 (<u>*C*</u>H-NH), 42.2 (lactam <u>*C*</u>H$_2$-NH), 35.5 (<u>*C*</u>H$_2$-aryl), 33.4 (lactam <u>*C*</u>H$_2$-CH), 31.6 (<u>*C*</u>H$_2$-CH$_2$-aryl), 28.9, 28.0 (lactam <u>*C*</u>H$_2$-CH$_2$NH and <u>*C*</u>H$_2$-CH$_2$CH), 22.3 (<u>*C*</u>H$_2$-CH$_3$) and 13.9 (<u>*C*</u>H$_3$); ESI *m/*z 100 %, 311.2 (MNa^+) and 22 %, 289.2 (MH^+); HR ESI *m/*z ($C_{17}H_{24}N_2O_2Na$ requires 311.1730) found 311.1732.

4.11, 4-Butylbenzoyl-(*R*)-3-amino-azepan-2-one

Lactam **2.66** (0.50 mL, 1.94 mmol) was dissolved in H_2O (10 mL) and cooled to 0°C. Acid chloride **4.02** (3 mmol) in dichloromethane (15 mL) and triethylamine (0.84 mL, 6 mmol) were added and stirred over night. H_2O (10 mL) was added and the reaction was extracted with dichloromethane (3 × 15 mL), the organic layer was washed with a pH 2 buffer (3 × 10 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 75:25 to 0:100) to give compound **4.11** as a white solid 0.28 g (48 %); *mp* 182-183 º C; $[\alpha]^{30}_D$ (c = 0.676, $CHCl_3$) -52.86; υ_{max}/cm^{-1}: 3204 (N-H), 2928 (saturated C-H), 1677, 1641 (C=O) and 1544 (aromatic); δ_H (400 MHz, $CDCl_3$) 7.74 (d, 2H, *J* 8, C<u>*H*</u>-CCO), 7.62 (d, 1H, *J* 5.5, N<u>*H*</u>-CH), 7.21 (d, 2H, *J* 8, C<u>*H*</u>-CBu), 6.69 (br.t, 1H, *J* 6.5, N<u>*H*</u>-CH$_2$), 4.69 (ddd, 1H, *J* 11, 6, 2 C<u>*H*</u>), 3.36-3.21 (m, 2H, lactam C<u>*H*</u>$_2$-NH), 2.62 (t, 2H, *J* 8, C<u>*H*</u>$_2$-aryl), 2.20 (br.d, 1H, *J* 13, lactam equatorial C<u>*H*</u>$_2$-CH), 2.05-1.98 (br.d, 1H, lactam equatorial C<u>*H*</u>$_2$-CH$_2$CH), 1.94-1.81 (m, 2H, lactam

equatorial C$\underline{H_2}$-CH_2NH and axial C$\underline{H_2}$-CH_2CH), 1.61-1.37 (m, 4H, *J* 7.5, C$\underline{H_2}$-CH_2-aryl, lactam axial C$\underline{H_2}$-CH, lactam axial C$\underline{H_2}$-CH_2CH), 1.32 (sextet, CH_2-CH_3, *J* 7.5, C$\underline{H_2}$-CH_3) and 0.90 (t, 3H, *J* 7, C$\underline{H_3}$); δ_C (100 MHz, $CDCl_3$) 175.9, 166.3 (<u>C</u>=O), 146.9 (<u>C</u>-^{n}Bu), 131.6 (<u>C</u>-CO), 128.5 (C<u>H</u>-C-^{n}Bu), 127.1 (<u>C</u>H-CCO), 52.5 (<u>C</u>H-NH), 42.2 (lactam <u>C</u>H_2-NH), 35.5 (<u>C</u>H_2-aryl), 33.4 (lactam <u>C</u>H_2-CH), 31.7 (<u>C</u>H_2-CH_2-aryl), 28.9, 28.0 (lactam <u>C</u>H_2-CH_2NH and <u>C</u>H_2-CH_2CH), 22.3 (<u>C</u>H_2-CH_3) and 13.9 (<u>C</u>H_3); ESI *m/*z 100 %, 598.7 (M_2Na^+) and 75 %, 311.1 (MNa^+); HR ESI *m/*z ($C_{17}H_{24}N_2O_2Na$ requires 311.1730) found 311.1737.

4.12, 4-Butyl benzoyl-(*S*)-3-amino-tetrahydropyridin-2-one

Lactam **2.53** (20 mmol) was dissolved in H_2O (20 mL) and cooled to 0°C. Acid chloride **4.02** in dichloromethane and triethylamine (4.2 mL, 30 mmol) were added and stirred over night. H_2O (20 mL) was added and the reaction was extracted with dichloromethane (3 × 20 mL), the organic layer was washed with a pH 2 buffer (3 × 15 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate:MeOH 50:50:0 to 0:80:20) to give compound **4.12** as a white solid 0.45 g (16 %); *mp* 117-118 º C; $[\alpha]^{24}_D$ (c = 0.523, $CHCl_3$) +98.73; δ_H (400 MHz, $CDCl_3$) 7.70 (d, 2H, *J* 8, C<u>H</u>-CO), 7.36 (d, 1H, *J* 6, N<u>H</u>-CH), 7.17 (d, 2H, *J* 8, C<u>H</u>-C-^{n}Bu), 6.67 (br.s, 1H, N<u>H</u>-CH_2), 4.69 (dt, 1H, *J* 12, 6, C<u>H</u>), 3.36-3.29 (m, 2H, C<u>H</u>$_2$-NH), 2.60 (t, 3H, *J* 7.5, C<u>H</u>$_2$-aryl and lactam C<u>H</u>$_2$-CH), 1.93-1.86 (m, 2H, lactam C<u>H</u>$_2$-NH), 1.67-1.54 (m, 1H, C<u>H</u>$_2$-CH), 1.55 (quintet, 2H, *J* 7.5, C<u>H</u>$_2$-CH_2-aryl), 1.30 (sextet, 2H, *J* 7, H7.5, C<u>H</u>$_2$-CH_3) and 0.89 (t, 3H, *J* 7.5, C<u>H</u>$_3$); δ_C (100 MHz, $CDCl_3$) 172.2, 167.5 (<u>C</u>=O), 146.9 (<u>C</u>-^{n}Bu), 131.5 (<u>C</u>-CO), 128.5 (<u>C</u>H-C^{n}Bu), 127.2 (<u>C</u>H-CCO), 50.8 (<u>C</u>H-NH), 41.7 (lactam <u>C</u>H_2-NH), 35.5 (<u>C</u>H_2-aryl), 33.3 (<u>C</u>H_2-CH_2-aryl), 27.2 (lactam <u>C</u>H_2-CH), 22.3 (<u>C</u>H_2-CH_3),

21.1 (lactam <u>C</u>H_2-CH_2NH) and 13.9 (<u>C</u>H_3); ESI *m/*z 100 %, 297.2 (MNa^+) and 26 %, 275.2 (MH^+); HR ESI *m/*z ($C_{16}H_{22}N_2O_2Na$ requires 297.1573) found 297.1573;

4.13, 4-Butyl benzoyl-(*R*)-3-amino-tetrahydropyridin-2-one

Lactam **2.54** (10 mmol) was dissolved in H_2O (25 mL) and cooled to 0°C. Acid chloride **4.02** (8.5 mmol) in dichloromethane (30 mL) and triethylamine (4.2 mL, 30 mmol) were added and stirred over night. The reaction was extracted with dichloromethane (3 × 15 mL), the organic layer was washed with a pH 2 buffer (3 × 15 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate:MeOH 50:50:0 to 0:80:20) to give compound **4.13** as a white solid 1.10 g (40 %); *mp* 116-117 º C; $[\alpha]^{25}_D$ (c = 0.493, $CHCl_3$) -89.45; υ_{max}/cm^{-1}: 3319, 3245 (N-H), 2949 (saturated C-H), 1651, 1634 (C=O) and 1521 (aromatic); δ_H (400 MHz, $CDCl_3$) 7.72 (d, 2H, *J* 8, C<u>*H*</u>-CO), 7.25 (d, 1H, *J* 5.5, N<u>*H*</u>-CH), 7.20 (d, 2H, *J* 8, C<u>*H*</u>-C-^{n}Bu), 6.41 (br.s, 1H, N<u>*H*</u>-CH_2), 4.41 (dt, 1H, *J* 11.5, 5.5, C<u>*H*</u>), 3.37-3.32 (m, 2H, lactam C<u>*H*$_2$</u>-NH), 2.66 (ddt, 1H, *J* 13, 6, 4.5, lactam equatorial C<u>*H*$_2$</u>-CH), 2.62 (t, 3H, *J* 8, C<u>*H*$_2$</u>-aryl), 1.98-1.90 (m, 2H, lactam C<u>*H*$_2$</u>-CH_2NH), 1.67-1.53 (m, 3H, lactam axial C<u>*H*$_2$</u>-CH and C<u>*H*$_2$</u>-CH_2-aryl), 1.32 (quintet, 2H, *J* 7.5, C<u>*H*$_2$</u>-CH_3) and 0.90 (t, 3H, *J* 7, C<u>*H*$_3$</u>); δ_C (100 MHz, $CDCl_3$) 172.1, 167.6 (<u>*C*</u>=O), 147.0 (<u>*C*</u>-^{n}Bu), 131.5 (<u>*C*</u>-CO), 128.5 (<u>*C*</u>H-C^{n}Bu), 127.2 (<u>*C*</u>H-CCO), 51.0 (<u>*C*</u>H-NH), 41.7 (lactam <u>*C*</u>H_2-NH), 35.5 (<u>*C*</u>H_2-aryl), 33.3 (<u>*C*</u>H_2-CH_2-aryl), 27.2 (lactam <u>*C*</u>H_2-CH), 22.3 (<u>*C*</u>H_2-CH_3), 21.1 (lactam <u>*C*</u>H_2-CH_2NH) and 13.9 (<u>*C*</u>H_3); ESI *m/*z 100 %, 297.1 (MNa^+); HR ESI *m/*z ($C_{16}H_{22}N_2O_2Na$ requires 297.1573) found 297.1574.

4.14, 4-*tert*-Butylbenzoyl-(*S*)-3-amino-azepan-2-one

Lactam **2.65** (2.04 g, 12.44 mmol) was dissolved in H_2O (20 mL) and cooled to 0°C. Acid chloride **4.03** in dichloromethane and triethylamine (4.2 mL, 30 mmol) were added and stirred over night. H_2O (20 mL) was added and the reaction was extracted with dichloromethane (3 × 20 mL), the organic layer was washed with a pH 2 buffer (3 × 20 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by recrystallisation from chloroform and cold petroleum ether and washed with boiling ethyl acetate to give compound **4.14** as a white solid 1.44 g (50 %); *mp* 204-205 º C; $[\alpha]^{23}_D$ (c = 0.523, $CHCl_3$) +63.77; υ_{max}/cm^{-1}: 3210 (N-H), 2906 (saturated C-H), 1640 (C=O) and 1566 (aromatic); *Anal.* ($C_{17}H_{24}N_2O_2$ requires: C; 70.80, H; 8.39, N; 9.70) found: C; 70.74, H; 8.37, N; 9.70; δ_H (400 MHz, $CDCl_3$) 7.76 (d, 2H, *J* 8.5, C*H*-CO), 7.66 (d, 1H, *J* 6, N*H*-CH), 7.42 (d, 2H, *J* 8.5, C*H*-C-^{t}Bu), 6.00 (br.s, 1H, N*H*-CH_2), 4.67 (ddd, 1H, *J* 11, 6, 1.5, C*H*), 3.34-3.19 (m, 2H, lactam C*H*$_2$-NH), 2.18 (br.d, 1H, *J* 13, lactam equatorial C*H*$_2$-CH), 2.00 (br.d, 1H, *J* 12.5, lactam equatorial C*H*$_2$-CH_2CH), 1.92-1.78 (m, 2H, lactam equatorial C*H*$_2$-CH_2NH and axial C*H*$_2$-CH_2CH), 1.51 (q, 1H, *J* 13, lactam axial C*H*$_2$-CH), 1.33 (br.q, 1H, *J* 11, lactam axial C*H*$_2$-CH_2NH), and 1.29 (s, 3H, C(C*H*$_3$)$_3$); δ_C (100 MHz, $CDCl_3$) 176.0, 166.2 (*C*=O), 155.0 (*C*-C$(CH_3)_3$), 131.4 (*C*-CO), 127.9 (*C*H-C^{t}Bu), 125.4 (*C*H-CCO), 52.5 (*C*H), 42.1 (lactam *C*H_2-NH), 34.9 (*C*$(CH_3)_3$), 31.6 (lactam *C*H_2-CH), 31.2 (C(*C*$H_3)_3$) and 28.9, 28.0 (lactam *C*H_2-CH_2NH and *C*H_2-CH_2CH); ESI *m/*z 100 %, 598.8 (M_2Na^+) and 32 %, 289.2 (MH^+); HR ESI *m/*z ($C_{17}H_{24}N_2O_2Na$ requires 311.1730) found 311.1736.

4.15, 4- *tert*-Butylbenzoyl-(*R*)-3-amino-azepan-2-one

Lactam **2.66** (0.17 g, 1.04 mmol) was dissolved in H_2O (10 mL) and cooled to 0°C. Acid chloride **4.03** (1.04 mmol) in dichloromethane (15 mL) and triethylamine (0.43 mL, 3.12 mmol) were added was stirred over night. H_2O (20 mL) was added and the reaction was extracted with dichloromethane (3 × 20 mL), the organic layer was washed with a pH 2 buffer (3 × 20 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate:MeOH 50:50:0 to 0:80:20) to give compound **4.15** as a white solid 0.15 g (50 %); *mp* 200-201 º C; $[\alpha]^{27}_D$ (c = 0.23, $CHCl_3$) -46.16; υ_{max}/cm^{-1}: 3211 (N-H), 2933 (saturated C-H), 1644 (C=O) and 1566 (aromatic); δ_H (400 MHz, $CDCl_3$) 7.77 (d, 2H, *J* 8.5, C*H*-CO), 7.62 (d, 1H, *J* 5.5, N*H*-CH), 7.44 (d, 2H, *J* 8.5, C*H*-C-^{t}Bu), 6.29 (br.t, 1H, *J* 6, N*H*-CH_2), 4.70 (ddd, 1H, *J* 11, 6, 2, C*H*), 3.39-3.32 (m, 2H, lactam C*H*$_2$-NH), 2.22 (br.d, 1H, *J* 13, lactam equatorial C*H*$_2$-CH), 2.07-2.0 (m, 1H, lactam equatorial C*H*$_2$-CH_2CH), 1.95-1.83 (m, 2H, lactam equatorial C*H*$_2$-CH_2NH and axial C*H*$_2$-CH_2CH), 1.54 (q, 1H, *J* 13, lactam axial C*H*$_2$-CH), 1.42 (br.q, 1H, *J* 13, lactam axial C*H*$_2$-CH_2NH) and 1.32 (s, 3H, C(C*H*$_3$)$_3$); δ_C (100 MHz, $CDCl_3$) 175.8, 166.2 (*C*=O), 155.0 (*C*-C$(CH_3)_3$), 131.3 (*C*-CO), 126.9 (*C*H-C^{t}Bu), 125.5 (*C*H-CCO), 52.6 (*C*H), 42.3 (lactam *C*H_2-NH), 34.9 (*C*$(CH_3)_3$), 31.7 (lactam *C*H_2-CH), 31.2 (C(*C*H_3)$_3$) and 29.0, 28.0 (lactam C*H*$_2$-CH_2NH and C*H*$_2$-CH_2CH); ESI *m/*z 100 %, 599.3 (M_2Na^+) and 17 %, 311.1 (MNa^+); HR ESI *m/*z ($C_{17}H_{24}N_2O_2Na$ requires 311.1730) found 311.1727.

4.16, 4-*tert*-Butyl benzoyl-(*S*)-3-amino-tetrahydropyridin-2-one

Lactam **2.53** (33 mmol) was dissolved in H_2O (100 mL) and cooled to 0°C. Acid chloride **4.03** (20 mmol) in dichloromethane and triethylamine (6.3 mL, 45 mmol)

were added and stirred over night. The reaction was extracted with dichloromethane (3 × 20 mL), the organic layer was washed with a pH 2 buffer (3 × 20 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate:MeOH 50:50:0 to 0:80:20) to give compound **4.16** as a white solid 3.13 g (53 %) *mp* 195-196 °C; $[\alpha]^{23}_D$ (c = 0.515, $CHCl_3$) +82.52; υ_{max}/cm^{-1}: 3251 (N-H), 2959 (saturated C-H), 1683, 1648 (C=O) and 1558 (aromatic); δ_H (400 MHz, $CDCl_3$) 7.24 (d, 2H, *J* 8.5, C*H*-CO), 7.49 (d, 1H, *J* 6, N*H*-CH), 7.36 (d, 2H, *J* 8.5, C*H*-C-^{t}Bu), 6.94 (br.s, 1H, N*H*-CH_2), 4.62 (dt, 1H, *J* 11, 6, 1.5, C*H*), 3.31-3.26 (m, 2H, C*H*$_2$-NH), 2.52 (ddt, 1H, *J* 13, 6, 4.5, lactam equatorial C*H*$_2$-CH), 1.90-183 (m, 2H, lactam C*H*$_2$-CH_2NH), 1.63 (tt, 1H, *J* 12.5, 8.5, lactam axial C*H*$_2$-CH) and 1.27 (s, 9H, C(C*H*$_3$)$_3$); δ_C (100 MHz, $CDCl_3$) 172.3, 167.4 (*C*=O), 154.9 (*C*-C$(CH_3)_3$), 131.2 (*C*-CO), 127.0 (*C*H-C^{t}Bu), 126.7 (*C*H-CCO), 50.8 (*C*H), 41.6 (lactam *C*H_2-NH), 34.9 (*C*$(CH_3)_3$), 31.2 (C(*C*$H_3)_3$), 27.2 (lactam C*H*$_2$-CH) and 21.1 (lactam C*H*$_2$-CH_2NH); ESI *m/*z 100 %, 297.2 (MNa^+) and 38 %, 275.2 (MH^+). HR ESI *m/*z ($C_{16}H_{23}N_2O_2$ requires 275.1754) found 275.1752.

4.17, 4-*tert*-Butyl benzoyl-(*R*)-3-amino-tetrahydropyridin-2-one

Lactam **2.54** (15 mmol) was dissolved in H_2O (100 mL) and cooled to 0°C. Acid chloride **4.03** (10 mmol) in dichloromethane and triethylamine (4.2 mL, 30 mmol) were added and stirred over night. The reaction was extracted with dichloromethane (3 × 20 mL), the organic layer was washed with a pH 2 buffer (3 × 20 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate:MeOH 50:50:0 to 0:80:20) to give compound **4.17** as a white solid 1.03 g (38 %) *mp* 193-194 °C; $[\alpha]^{23}_D$ (c = 0.512, $CHCl_3$) -84.08; υ_{max}/cm^{-1}: 3247 (N-H), 2958 (saturated C-H), 1682, 1647 (C=O) and

1544 (aromatic); δ_H (400 MHz, $CDCl_3$) 7.72 (d, 2H, *J* 8.5, C*H*-CO), 7.49 (d, 1H, *J* 6, N*H*-CH), 7.36 (d, 2H, *J* 8.5, C*H*-C-^{t}Bu), 6.93 (br.s, 1H, N*H*-CH_2), 4.39 (dt, 1H, *J* 11.5, 6, C*H*), 3.31-3.26 (m, 2H, lactam C*H*$_2$-NH), 2.52 (ddt, 1H, *J* 12.5, 5.5, 4.5, lactam equatorial C*H*$_2$-CH), 1.90-1.87 (m, 2H, lactam C*H*$_2$-CH_2NH), 1.63 (tt, 1H, *J* 12.5, 8.5, lactam axial C*H*$_2$-CH) and 1.27 (s, 9H, C(C*H*$_3$)$_3$); δ_C (100 MHz, $CDCl_3$) 172.3, 167.4 (*C*=O), 154.9 (*C*-C(CH_3)$_3$), 131.2 (*C*-C=O), 127.0 (*C*H-C^{t}Bu), 125.3 (*C*H-CCO), 50.8 (*C*H), 41.6 (lactam *C*H_2-NH), 34.9 (*C*(CH_3)$_3$), 31.2 (C(*C*H_3)$_3$), 27.2 (lactam *C*H_2-CH) and 21.1 (lactam *C*H_2-CH_2NH); ESI *m/*z 19 %, 297.2 (MNa^+) and 13 %, 275.2 (MH^+); HR ESI *m/*z ($C_{16}H_{22}N_2O_2$ requires 297.1573) found 275.1750.

4.18, 4-Hexylbenzoyl-(*S*)-3-amino-azepan-2-one

Lactam **2.65** (0.95 g, 5.79 mmol) was dissolved in H_2O (15 mL) and cooled to 0°C. 4-Hexylbenzoyl chloride **4.04** (1.2 mL, 5 mmol) in dichloromethane and triethylamine (2.1 mL, 15 mmol) were added and the reaction was stirred over night. H_2O (20 mL) was added and the reaction was extracted with dichloromethane (3 × 20 mL), the organic layer was washed with a pH 2 buffer (3 × 15 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 50:50:0 to 0:80:20) to give compound **4.18** as a white solid 0.76 g (48 %); *mp* 167-168 º C; $[\alpha]^{25}_D$ (c = 0.507, $CHCl_3$) +60.06; υ_{max}/cm^{-1}: 3244 (N-H), 2956 (saturated C-H), 1658, 1644 (C=O) and 1543 (aromatic); *Anal.* ($C_{19}H_{28}N_2O_2$ requires: C; 72.12, H; 8.92, N; 8.85) found: C; 72.11, H; 8.92, N; 7.82; δ_H (400 MHz, $CDCl_3$) 7.74 (d, 2H, *J* 8, C*H*-CO), 7.61 (d, 1H, *J* 6, N*H*-CH), 7.21 (d, 2H, *J* 8, C*H*-CHex), 6.54 (br.t, 1H, *J* 6, N*H*-CH_2), 4.69 (ddd, 1H, *J* 11, 6, 1.5, C*H*), 3.37-3.22 (m, 2H, lactam C*H*$_2$-NH), 2.62 (t, 2H, *J* 7.5, C*H*$_2$-aryl), 2.21 (d, 1H, *J* 13, lactam equatorial C*H*$_2$-CH), 2.03 (dt, 1H, *J* 14, 3.5,

lactam equatorial C*H*$_2$-CH$_2$CH), 1.95-1.82 (m, 2H, lactam equatorial C*H*$_2$-CH$_2$NH and axial C*H*$_2$-CH$_2$CH), 1.64-1.49 (m, 3H, lactam axial C*H*$_2$-CH and C*H*$_2$-CH$_2$-aryl), 1.41 (q, 1H, *J* 13, lactam axial C*H*$_2$-CH$_2$NH) 1.33-1.23 (m, 6H, CH$_3$-C*H*$_2$-C*H*$_2$-C*H*$_2$) and 0.86 (t, 3H, *J* 7, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 175.9, 166.3 (*C*=O), 147.0 (*C*-Hex), 131.6 (*C*-CO), 128.5 (phenyl *C*H-CHex), 127.1 (phenyl *C*H-CO), 52.6 (*C*H), 42.2 (lactam *C*H$_2$-NH), 35.8 (*C*H$_2$-aryl), 31.7 (lactam *C*H$_2$-NH), 31.2 (*C*H$_2$-CH$_2$-aryl), 29.0, 28.9, 28.0, (lactam *C*H$_2$-CH$_2$NH, *C*H$_2$-CH$_2$CH and *C*H$_2$-CH$_2$CH$_2$-aryl, *C*H$_2$-CH$_2$CH$_3$), 22.6 (*C*H$_2$-CH$_3$) and 14.1 (*C*H3); ESI *m/*z 43 %, 317.2 (MH^+), 6% 339.2 (MNa^+) and 6 %, 654.7 (M_2Na^+); HR ESI *m/*z ($C_{19}H_{28}N_2O_2Na$ requires 339.2043) found 339.2050.

4.19, 4-Hexylbenzoyl-(*R*)-3-amino-azepan-2-one

Lactam **2.66** (0.27 g, 1.62 mmol) was dissolved in H_2O (15 mL) and cooled to 0°C. 4-Hexylbenzoyl chloride **4.04** (0.23 mL, 1.04 mmol) in dichloromethane and triethylamine (0.42 mL, 3.12 mmol) were added and stirred over night. H_2O (20 mL) was added and the reaction was extracted with dichloromethane (3 × 20 mL), the organic layer was washed with a pH 2 buffer (3 × 15 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 50:50:0 to 0:80:20) to give compound **4.19** as a white solid 0.23 g (69 %); *mp* 168-169 º C; $[\alpha]^{27}_D$ (c = 0.518, $CHCl_3$) -52.77; υ_{max}/cm^{-1}: 3201 (N-H), 2926 (saturated C-H), 1678, 1640 (C=O) and 1543 (aromatic); δ_H (400 MHz, $CDCl_3$) 7.74 (d, 2H, *J* 8.5, C*H*-CO), 7.61 (d, 1H, *J* 6, N*H*-CH), 7.22 (d, 2H, *J* 8.5, C*H*-CHex), 6.36 (br.t, 1H, *J* 6, N*H*-CH$_2$), 4.70 (ddd, 1H, *J* 11, 5, 2.5, C*H*), 3.37-3.22 (m, 2H, lactam C*H*$_2$-NH), 2.62 (t, 2H, *J* 8, C*H*$_2$-aryl), 2.22 (d, 1H, *J* 14, lactam equatorial C*H*$_2$-CH), 2.06-1.99 (m, 1H, lactam equatorial C*H*$_2$-CH$_2$CH), 1.95-1.83

(m, 2H, lactam equatorial C*H*$_2$-CH$_2$NH and axial C*H*$_2$-CH$_2$CH), 1.62-1.51 (m, 3H, lactam axial C*H*$_2$-CH and C*H*$_2$-CH$_2$-aryl), 1.39 (q, 1H, *J* 13, lactam axial C*H*$_2$-CH$_2$NH), 1.33-1.24 (m, 6H, CH$_3$-C*H*$_2$-CH$_2$-C*H*$_2$) and 0.86 (t, 3H, *J* 7.5, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 175.8, 166.3 (*C*=O), 150.0 (*C*-Hex), 131.6 (*C*-CO), 128.5 (phenyl *C*H-CHex), 127.1 (*C*H-CCO), 52.6 (*C*H-NH), 42.3 (lactam *C*H$_2$-NH), 35.9 (*C*H$_2$-aryl), 31.7 (lactam *C*H$_2$-CH), 31.2 (*C*H$_2$-CH$_2$-aryl), 29.0, 28.9, 28.0, (lactam C*H*$_2$-CH$_2$NH and *C*H$_2$-CH$_2$CH, and *C*H$_2$-*C*H$_2$-CH$_2$-CH$_3$), 22.6 (*C*H$_2$-CH$_3$) and 14.1 (*C*H$_3$); ESI *m/*z 100 %, 655.3 (M_2Na^+) and 45 % 339.1 (MNa^+); HR ESI *m/*z ($C_{19}H_{28}N_2O_2Na^+$ requires 339.2043) found 339.2049.

4.20, 4-Hexylbenzoyl-(*S*)-3-amino-tetrahydropyridin-2-one

Lactam **2.53** (10 mmol) was dissolved in H_2O (35 mL) and cooled to 0°C. 4-Hexylbenzoyl chloride **4.04** (1.2 mL, 5 mmol) in dichloromethane and triethylamine (2.1 mL, 15 mmol) were added and stirred over night. The reaction was extracted with dichloromethane (3 × 15 mL), the organic layer was washed with a pH 2 buffer (3 × 15 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 50:50:0 to 0:80:20) to give compound **4.20** as a white solid 0.95 g (63 %); *mp* 118-119 º C; $[\alpha]^{23}_D$ (c = 0.511, $CHCl_3$) +79.55; υ_{max}/cm^{-1}: 3338, 3247 (N-H), 2921 (saturated C-H), 1656, 1637 (C=O) and 1562 (aromatic); δ_H (400 MHz, $CDCl_3$) 7.70 (d, 2H, *J* 8, C*H*-CO), 7.38 (d, 1H, *J* 6, N*H*-CH), 7.17 (d, 2H, *J* 8, C*H*-CHex), 6.81 (br.s, 1H, N*H*-CH$_2$), 4.39 (dt, 1H, *J* 11.5, 6, C*H*), 3.33-3.26 (m, 2H, lactam C*H*$_2$-NH), 2.58 (t, 2H, *J* 7.5, lactam C*H*$_2$-aryl), 2.57-2.52 (m, 1H, lactam equatorial C*H*$_2$-CH), 1.92-1.84 (m, 2H, lactam C*H*$_2$-CH$_2$NH), 1.67-1.52 (m, 3H, lactam axial C*H*$_2$-CH and C*H*$_2$-CH$_2$-aryl), 1.29-1.23 (m, 6H, CH$_3$-C*H*$_2$-C*H*$_2$-C*H*$_2$) and 0.84 (t, 3H, *J* 7.5, C*H*$_3$); δ_C (100 MHz,

$CDCl_3$) 172.3, 167.5 (*C*=O), 146.9 (*C*-Hex), 131.5 (*C*-CO), 128.4 (*C*H-CHex), 127.2 (*C*H-CCO), 50.8 (*C*H-NH), 41.6 (lactam *C*H_2-NH), 35.8 (*C*H_2-aryl), 31.7 (lactam *C*H_2-CH), 31.2 (*C*H_2-CH_2-aryl), 28.9 (*C*H_2-CH_2-CH_2-aryl), 27.2, (*C*H_2-CH_2CH_3), 22.6 (*C*H_2-CH_3), 21.1 (lactam *C*H_2-CH_2NH) and 14.1 (*C*H_3); ESI *m/*z 100 %, 325.2 (MNa^+) and 37% 303.2 (MH^+); HR ESI *m/*z ($C_{18}H_{26}N_2O_2Na$ requires 325.1886) found 325.1883.

4.21, 4-Hexylbenzoyl-(*R*)-3-amino-tetrahydropyridin-2-one

Lactam **2.54** (10 mmol) was dissolved in H_2O (20 mL) and cooled to 0°C. 4-Hexylbenzoyl chloride **4.04** (1.2 mL, 5 mmol) in dichloromethane and triethylamine (2.1 mL, 15 mmol) was added and stirred over night. The reaction was extracted with dichloromethane (3 × 15 mL), the organic layer was washed with a pH 2 buffer (3 × 15 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 50:50:0 to 0:80:20) to give compound **4.21** as a white solid 0.53 g (35 %); *mp* 117-118 ° C; υ_{max}/cm^{-1}: 3328, 3240 (N-H), 2954 (saturated C-H), 1651, 1635 (C=O) and 1533 (aromatic); $[\alpha]^{25}_D$ (c = 0.496, $CHCl_3$) -81.17; $_H$ (400 MHz, $CDCl_3$) 7.71 (d, 2H, *J* 8, C*H*-CO), 7.28 (d, 1H, *J* 5, N*H*-CH), 7.19 (d, 2H, *J* 8, C*H*-CHex), 6.52 (br.s, 1H, N*H*-CH_2), 4.41 (dt, 1H, *J* 11.5, 5.5, C*H*), 3.35-3.31 (m, 2H, lactam C*H*$_2$-NH), 2.67-2.60 (m, 1H, lactam equatorial C*H*$_2$-CH), 2.61 (t, 2H, *J* 7.5, C*H*$_2$-aryl), 2.00-1.89 (m, 2H, C*H*$_2$-CH_2NH), 1.67-1.54 (m, 3H, equatorial C*H*$_2$-CH and C*H*$_2$-CH_2-aryl), 1.32-1.23 (m, 6H, C*H*$_2$-C*H*$_2$-C*H*$_2$-CH_3) and 0.85 (t, 3H, *J* 7, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 172.1, 167.6 (*C*=O), 147.0 (*C*-Hex), 131.5 (*C*-CO), 128.5 (*C*H-CHex), 127.2 (*C*H-CCO), 50.9 (*C*H), 41.7 (lactam *C*H_2-NH), 35.8 (*C*H_2-aryl), 31.7 (lactam *C*H_2-CH), 31.2 (*C*H_2-CH_2-aryl), 28.9 (*C*H_2-CH_2CH_2-aryl), 27.2, (*C*H_2-CH_2-CH_3), 22.6 (*C*H_2-CH_3),

21.1 (lactam <u>C</u>H_2-CH_2NH) and 14.1 (<u>C</u>H_3); ESI *m/*z 100 %, 325.2 (MNa^+) and 33% 303.2 (MH^+); HR ESI *m/*z ($C_{18}H_{26}N_2O_2Na$ requires 325.1886) found 325.1888.

4.22, 4-Octylbenzoyl-(*S*)-3-amino-azepan-2-one

Lactam **2.65** (0.91 g, 5.55 mmol) was dissolved in H_2O (15 mL) and cooled to 0°C. Acid chloride **4.05** in dichloromethane and triethylamine (0.84 mL, 6 mmol) were added and the reaction was stirred over night. H_2O (20 mL) was added and the reaction was extracted with dichloromethane (3 × 20 mL), the organic layer was washed with a pH 2 buffer (3 × 15 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 50:50:0 to 0:80:20) to give compound **4.22** as a white solid 0.087 g (4 %); *mp* 159-160 º C; $[\alpha]^{25}_D$ (c = 0.124, $CDCl_3$) +68.01; υ_{max}/cm^{-1}: 3204 (N-H), 2923 (saturated C-H), 1637 (amide C=O) and 1544 (aromatic); δ_H (400 MHz, $CDCl_3$) 7.73 (d, 2H, *J* 8, C<u>*H*</u>-CO), 7.64 (d, 1H, *J* 5.5, N<u>*H*</u>-CH), 7.20 (d, 2H, *J* 8, C<u>*H*</u>-C-Oct), 6.94 (br.t, 1H, *J* 6, N<u>*H*</u>-CH_2), 4.68 (dd, 1H, *J* 11, 6, C<u>*H*</u>), 3.35-3.20 (m, 2H, lactam C<u>*H*$_2$</u>-NH), 2.60 (t, 2H, *J* 7.5, C<u>*H*$_2$</u>-aryl), 2.19 (d, 1H, *J* 13, lactam equatorial C<u>*H*$_2$</u>-CH), 2.00 (br.d, 1H, equatorial C<u>*H*$_2$</u>-CH_2NH), 1.92-1.79 (m, 2H, lactam axial C<u>*H*$_2$</u>-CH_2NH), 1.62-1.46 (m, 3H, lactam axial C<u>*H*$_2$</u>-CH and C<u>*H*$_2$</u>-CH_2-aryl), 1.38 (q, 1H, *J* 11.5, lactam axial C<u>*H*$_2$</u>-CH_2NH), 1.30-1.19 (m, 10H, H7, H8, H9, H10 & H11) and 0.84 (t, 3H, *J* 7.5, H12); δ_C (100 MHz, $CDCl_3$) 176.0, 166.3 (<u>*C*</u>=O), 146.9 (<u>*C*</u>-Oct), 131.6 (<u>*C*</u>-CO), 128.5 (<u>*C*</u>H-COct), 127.1 (<u>*C*</u>H-CCO), 52.5 (<u>*C*</u>H), 42.1 (lactam <u>C</u>H_2-NH), 35.8 (<u>*C*</u>H_2-aryl), 31.9 (lactam <u>C</u>H_2-CH), 31.6 (<u>*C*</u>H_2-CH_2-aryl), 31.2 (<u>*C*</u>H_2-CH_2-CH_2-aryl), 29.4, 29.3, 28.9, 28.0, (lactam <u>*C*</u>H_2-CH_2NH and <u>*C*</u>H_2-CH_2CH and <u>*C*</u>H_2-<u>*C*</u>H_2-$CH_2CH_2CH_3$), 22.7 (<u>*C*</u>H_2CH_3) and 14.1 (<u>*C*</u>H_3); ESI *m/*z 100 %, 345.2 (MH^+)

and 9 %, 710.8 (M_2Na^+); HR ESI *m/*z ($C_{21}H_{32}N_2O_2Na$ requires 367.2356) found 367.2361.

4.23, 4-Octylbenzoyl-(*R*)-3-amino-azepan-2-one

Lactam **2.66** (0.112 g, 0.44 mmol) was dissolved in H_2O (5 mL) and cooled to 0°C. Acid chloride **4.05** (2 mmol) in dichloromethane (10 mL) and triethylamine (0.21 mL, 1.5 mmol) were added and the reaction was stirred over night. H_2O (10 mL) was added and the reaction was extracted with dichloromethane (3 × 15 mL), the organic layer was washed with a pH 2 buffer (3 × 15 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 50:50:0 to 0:90:10) to give compound **4.23** as a white solid 0.018 g (10 %); *mp* 157-158 º C; $[\alpha]^{27}_D$ (c = 0.12, $CDCl_3$) -72.36; υ_{max}/cm^{-1}: 3206 (N-H), 2922 (saturated C-H), 1642 (amide C=O) and 1544 (aromatic); δ_H (400 MHz, $CDCl_3$) 7.74 (d, 2H, *J* 8, C*H*-CO), 7.61 (d, 1H, *J* 5.5, N*H*-CH), 7.22 (d, 2H, *J* 8, C*H*-C-Oct), 6.48 (br.t, 1H, *J* 5.5, N*H*-CH_2), 4.70 (ddd, 1H, *J* 11, 6, 2, C*H*), 3.37-3.22 (m, 2H, lactam C*H*$_2$-NH), 2.62 (t, 2H, *J* 7, C*H*$_2$-aryl), 2.21 (d, 1H, *J* 13, lactam equatorial C*H*$_2$-CH), 2.06-1.99 (m, 1H, lactam equatorial C*H*$_2$-CH_2CH), 1.95-1.87 (m, 3H, lactam axial C*H*$_2$-CH_2NH and C*H*$_2$-CH_2CH), 1.63-1.50 (m, 3H, lactam axial C*H*$_2$-CH and C*H*$_2$-CH_2-aryl), 1.40 (q, 1H, *J* 12, lactam equatorial C*H*$_2$-CH_2NH) 1.30-1.20 (m, 11H, C*H*$_2$-CH_2-aryl and C*H*$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$-CH_3) and 0.86 (t, 3H, *J* 7, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 175.9, 166.3 (*C*=O), 147.0 (*C*-Oct), 131.6 (*C*-CO), 128.5 (*C*H-COct), 127.1 (*C*H-CCO), 52.6 (*C*H), 42.2 (lactam *C*H_2-NH), 35.9 (*C*H_2-aryl), 31.9 (lactam *C*H_2-CH), 31.7 (*C*H_2-CH_2-aryl), 31.2 (*C*H_2-CH_2-CH_2-aryl), 29.4, 29.2, 29.0, 28.9, 28.0, (lactam *C*H_2-CH_2NH and *C*H_2-CH_2CH and *C*H_2-*C*H_2-*C*H_2-

$\underline{C}H_2$-CH_3), 22.7 ($\underline{C}H_2$-CH_3) and 14.1 ($\underline{C}H_3$); ESI *m/*z 100 %, 367.2 (MNa^+) and 21 %, 710.8 (M_2Na^+); HR ESI *m/*z ($C_{21}H_{32}N_2O_2Na^+$ requires 367.2356) found 367.2349.

4.24, 4-Octylbenzoyl-(*S*)-3-amino-tetrahydropyridin-2-one

Lactam **2.53** (10 mmol) was dissolved in H_2O (35 mL) and cooled to 0°C. Acid chloride **4.05** (2 mmol) in dichloromethane and triethylamine (0.85 mL, 6 mmol) were added and stirred over night. The reaction was extracted with dichloromethane (3 × 10 mL), the organic layer was washed with a pH 2 buffer (3 × 10 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 50:50:0 to 0:80:20) to give compound **4.24** as a white solid 0.55 g (83 %); *mp* 122-123 º C; $[\alpha]^{23}_D$ (c = 0.509, $CHCl_3$) +75.74; υ_{max}/cm^{-1}: 3240 (N-H), 2921 (saturated C-H), 1653, 1615 (C=O) and 1563 (aromatic); δ_H (400 MHz, $CDCl_3$) 7.71 (d, 2H, *J* 8, C$\underline{H}$-CO), 7.26 (d, 1H, *J* 5.5, N$\underline{H}$-CH), 7.19 (d, 2H, *J* 8, C$\underline{H}$-C-Oct), 6.47 (br.s, 1H, N$\underline{H}$-CH_2), 4.41 (dt, 1H, *J* 11.5, 5.5, C$\underline{H}$), 3.33-3.27 (m, 2H, lactam C$\underline{H}_2$-NH), 2.84-2.58 (m, 1H, *J* 13, lactam equatorial C$\underline{H}_2$-CH), 2.60 (t, 2H, *J* 7.5, C$\underline{H}_2$-aryl), 1.97-1.90 (m, 2H, C$\underline{H}_2$-CH_2NH), 1.67-1.54 (m, 3H, lactam axial C$\underline{H}_2$-CH and C$\underline{H}_2$-CH_2-aryl), 1.29-1.23 (m, 10H, C$\underline{H}_2$-C$\underline{H}_2$-C$\underline{H}_2$-C$\underline{H}_2$-C$\underline{H}_2$-CH_3) and 0.86 (t, 3H, *J* 7, C$\underline{H}_3$); δ_C (100 MHz, $CDCl_3$) 172.1, 167.6 ($\underline{C}$=O), 147.0 ($\underline{C}$-Oct), 131.5 ($\underline{C}$-C=O), 128.5 ($\underline{C}$H-C-Oct), 127.2 ($\underline{C}$H-CO), 50.9 ($\underline{C}$H), 41.7 (lactam $\underline{C}H_2$-NH), 35.8 ($\underline{C}H_2$-aryl), 31.9 ($\underline{C}H_2$-CH_2-aryl), 31.2 (lactam $\underline{C}H_2$-CH), 29.4 ($\underline{C}H_2$-CH_2-CH_2-aryl), 29.3 ($\underline{C}H_2$-CH_2-CH_2-CH_2-CH_2-aryl), 27.2 ($\underline{C}H_2$-CH_2CH_3), 22.3 ($\underline{C}H_2$-CH_3), 21.1 (lactam $\underline{C}H_2$-CH_2NH) and 14.1 ($\underline{C}H_3$); ESI *m/*z 100 %, 353.2 (MNa^+) and 47 %, 331.2 (MH^+); HR ESI *m/*z ($C_{20}H_{30}N_2O_2Na$ requires 353.2199) found 353.2198.

4.25, 4-Octylbenzoyl-(*R*)-3-amino-tetrahydropyridin-2-one

Lactam **2.54** (5 mmol) was dissolved in H_2O (25 mL) and cooled to 0°C. Acid chloride **4.05** (2 mmol) in dichloromethane and triethylamine (0.85 mL, 6 mmol) were added and stirred over night. The reaction was extracted with dichloromethane (3 × 10 mL), the organic layer was washed with a pH 2 buffer (3 × 10 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 50:50:0 to 0:80:20) to give compound **4.24** as a white solid 0.16 g (25 %); *mp* 122-123 º C; $[\alpha]^{23}_D$ (c = 0.485, $CHCl_3$) -77.80; υ_{max}/cm^{-1}: 3250 (N-H), 2955 (saturated C-H), 1653 (C=O) and 1540 (aromatic); δ_H (400 MHz, $CDCl_3$) 7.71 (d, 2H, *J* 8.5, C*H*-CO), 7.23 (d, 1H, *J* 6.5, N*H*-CH), 7.20 (d, 2H, *J* 8.5, C*H*-C-Oct), 6.34 (br.s, 1H, N*H*-CH_2), 4.41 (dt, 1H, *J* 11.5, 5.5, C*H*), 3.37-3.33 (m, 2H, lactam C*H*$_2$-NH), 2.68 (ddt, 1H, *J* 13, 5.5, 4.5, lactam equatorial C*H*$_2$-CH), 2.61 (t, 2H, *J* 7.5, C*H*$_2$-aryl), 1.98-1.91 (m, 2H, lactam C*H*$_2$-CH_2NH), 1.67-1.55 (m, 3H, lactam axial C*H*$_2$-CH and C*H*$_2$-CH_2-aryl), 1.31-1.22 (m, 10H, C*H*$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$-CH_3) and 0.86 (t, 3H, *J* 7, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 172.1, 167.6 (*C*=O), 147.0 (*C*-Oct), 131.5 (*C*-C=O), 128.5 (*C*H-COct), 127.2 (*C*H-CCO), 51.0 (*C*H), 41.7 (lactam *C*H_2-NH), 35.8 (*C*H_2-aryl), 31.9 (*C*H_2-CH_2-aryl), 31.2 (lactam *C*H_2-CH), 29.4 (*C*H_2-CH_2CH_2-aryl), 29.3 (*C*H_2-*C*$H_2CH_2CH_2CH_2$-aryl), 27.2 (*C*H_2-CH_2CH_3), 22.7 (*C*H_2CH_3), 21.1 (lactam *C*H_2-CH_2NH) and 14.1 (*C*H_3); ESI *m/*z 39 %, 353.2 (MNa^+), 19 %, 331.2 (MH^+) and 14 %, 682.7 (M_2Na^+); HR ESI *m/*z ($C_{20}H_{30}N_2O_2H^+$ requires 331.2380) found 331.2381.

6.4.2 4-Sulfonyl Compounds

4.26, 4-Ethylbenzenesulfonylchloride

4-Ethylbenzoic acid (1.5 mL, 10 mmol) was dissolved in dichloromethane (20 mL), oxalyl chloride (1.3 mL, 15 mmol) and DMF (1 drop) were added and the reaction was stirred for 4 hours. The organic solvents were removed *in vacuo* to give compound **4.26** was not isolated and used in the next stage.

4.28, 4-Octylbenzenesulfonyl chloride

4-Octylbenzoic acid (1.25 g, 1.34 mmol) was dissolved in dichloromethane (25 mL), oxalyl chloride (0.12 mL, 1.3 mmol), DMF (1 drop) and tetrabutylammonium chloride (0.037 g, 0.134 mmol) were added and the reaction was stirred for 4 hours. The organic solvents were removed *in vacuo* to give compound **4.28** which was not isolated and used in the next stage**.**

4.29, 4-Ethylbenzenesulfonyl-(*S*)-3-amino-azepan-2-one

Lactam **2.65** (0.55 g, 3.3 mmol) was dissolved in H_2O (20 mL) and cooled to 0°C. Sulfonyl chloride **4.26** (5 mmol) in dichloromethane (30 mL) and triethylamine (1.3 mL, 9.0 mmol) were added and stirred over night. The reaction was extracted with dichloromethane and washed with pH 2 buffer (3 × 20 mL) and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 75:25 to 0:100) to give compound **4.29** as a white solid (0.17 g, 19 %); *mp* 159-160 ° C; $[\alpha]^{25}_{D}$ (c = 0.235, $CHCl_3$) +128.79; υ_{max}/cm^{-1}: 3389, 3228 (N-H), 2927 (saturated C-H), 1660 (amide C=O) and 1362, 1163 (SO_2); *Anal.* ($C_{14}H_{20}N_2O_3S$

requires: C; 56.73, H; 6.80, N; 9.45) found: C; 56.65, H; 6.80, N; 9.44; δ_H (400 MHz, $CDCl_3$) 7.72 (d, 2H, *J* 8, C*H*-CSO_2), 7.28 (d, 2H, *J* 8, C*H*-CEt), 6.40 (br.t, 1H, *J* 6, N*H*-CH_2), 6.18 (d, 1H, *J* 5, N*H*-CH), 3.83-3.74 (m, 1H, C*H*), 3.19-3.11 (m, 1H, lactam CH_2-NH), 3.03 (ddd, 1H, *J* 16, 11.5, 5.5, lactam CH_2-NH), 2.08 (q, 2H, *J* 8, CH_2-aryl), 2.15-2.09 (m, 1H, lactam CH_2-CH), 2.0-1.95 (m, 1H, lactam CH_2-CH_2NH), 1.80-1.77 (m, 1H, lactam CH_2-CH_2CH), 1.66-1.52 (m, 2H, lactam CH_2-CH_2CH and CH_2-CH), 1.37-1.26 (m, 1H, lactam CH_2-CH_2NH) and 1.23 (t, 3H, *J* 8, CH_3); δ_C (100 MHz, $CDCl_3$) 174.4 (*C*=O), 149.5 (*C*-Et), 137.2 (*C*-SO_2), 129.1 (*C*H-CEt), 126.2 (*C*H-CSO_2), 55.4 (*C*H-NH), 42.2 (lactam *C*H_2-NH), 33.4 (lactam *C*H_2-CH), 28.8 (*C*H_2-aryl), 28.6 (lactam *C*H_2-CH_2NH), 28.0 (lactam *C*H_2-CH_2CH) and 15.1 (*C*H_3); ESI *m/*z 100 %, 319.1 (MNa^+) and 58 %, 614.6 (M_2Na^+); HR ESI *m/*z ($C_{114}H_{20}N_2O_3SNa^+$ requires 319.1087) found 319.1085.

4.30, 4-Ethylbenzenesulfonyl-(*S*)-3-amino-tetrahydropyridin-2-one

Lactam **2.53** (4.8 mmol) was dissolved in H_2O (20 mL) and cooled to 0°C. Sulfonyl chloride **4.26** (1.50 mL, 10.9 mmol) in dichloromethane (20 mL) and triethylamine (4.2 mL, 30 mmol) were added and stirred over night. The dichloromethane was removed *in vacuo* and the product was dissolved in ethyl acetate and washed with pH 2 buffer (3 × 20 mL) and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 75:25 to 0:100) to give compound **4.30** as a white solid (0.67 g, 24 %); *mp* 142-143 ° C; $[\alpha]^{26}_D$ (c = 0.54, $CHCl_3$) +130.12; υ_{max}/cm^{-1}: 3212, 3105 (N-H), 2938, 2876 (saturated C-H), 1656 (C=O), 1597 (aromatic) and 1323, 1131 (SO_2); δ_H (400 MHz, $CDCl_3$) 7.79 (d, 2H, *J* 8, C*H*-CSO_2), 7.31 (d, 2H, *J* 8, C*H*-CEt), 6.01 (br.s, 1H, N*H*-CH_2), 5.89 (d, 1H, *J* 2, N*H*-CH), 3.50-3.45 (m, 1H, C*H*), 3.30-3.24 (m, 2H, lactam CH_2-NH), 2.70 (q, 2H, *J*

7, C<u>*H*</u>$_2$-aryl), 2.50-2.45 (m, 1H, lactam C<u>*H*</u>$_2$-CH), 1.95-1.85 (m, 1H, lactam C<u>*H*</u>$_2$-CH_2NH), 1.84-1.68 (m, 2H, lactam C<u>*H*</u>$_2$-CH_2NH and C<u>*H*</u>$_2$-CH), and 1.24 (t, 3H, *J* 7, C<u>*H*</u>$_3$); δ_C (100 MHz, $CDCl_3$) 169.9 (<u>*C*</u>=O), 149.3 (<u>*C*</u>-$C(CH_3)_3$), 136.2 (<u>*C*</u>-SO_2), 128.6 (<u>*C*</u>H-CEt), 127.5 (<u>*C*</u>H-CSO_2), 53.3 (<u>*C*</u>H), 41.9 (lactam <u>*C*</u>H_2-NH), 28.8 (<u>*C*</u>H_2-aryl), 28.5 (lactam <u>*C*</u>H_2-CH), 20.8 (lactam <u>*C*</u>H_2-CH_2NH) and 15.1 (<u>*C*</u>H_3); ESI *m/*z 100 %, 586.6 (M_2Na^+) and 59 %, 305.0 (MNa^+); HR ESI *m/*z ($C_{13}H_{18}N_2O_3SNa^+$ requires 305.0930) found 305.0931.

4.31, 4-Ethylbenzenesulfonyl-(*R*)-3-amino-tetrahydropyridin-2-one

Lactam **2.54** (4 mmol) was dissolved in H_2O (20 mL) and cooled to 0°C. Sulfonyl chloride **4.26** (4 mmol) in dichloromethane (25 mL) and triethylamine (1.7 mL, 12 mmol) were added and the reaction was stirred over night. The reaction was extracted with dichloromethane and washed with pH 2 buffer (3 × 20 mL) and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate:MeOH 75:25:0 to 0:90:10) to give compound **4.31** as a white solid (0.25 g, 21 %); δ_H (400 MHz, $CDCl_3$) 7.80 (d, 2H, *J* 8, C<u>*H*</u>-CSO_2), 7.32 (d, 2H, *J* 8, C<u>*H*</u>-CEt), 6.09 (br.s, 1H, N<u>*H*</u>-CH_2), 5.90 (d, 1H, *J* 2, N<u>*H*</u>-CH), 3.50-3.45 (m, 1H, C<u>*H*</u>), 3.28-3.23 (m, 2H, lactam C<u>*H*</u>$_2$-NH), 2.69 (q, *J* 7.5, 2H, C<u>*H*</u>$_2$-aryl), 2.49-2.45 (m, 1H, lactam C<u>*H*</u>$_2$-CH), 1.95-1.90 (m, 1H, lactam C<u>*H*</u>$_2$-CH_2NH), 1.82-1.71 (m, 2H, lactam C<u>*H*</u>$_2$-CH_2NH and C<u>*H*</u>$_2$-CH) and 1.24 (t, 3H, C<u>*H*</u>$_3$); δ_C (100 MHz, $CDCl_3$) 170.0 (<u>*C*</u>=O), 149.7 (<u>*C*</u>-Et), 136.2 (<u>*C*</u>-SO_2), 129.1 (<u>*C*</u>H-CEt), 128.6 (<u>*C*</u>H-CSO_2), 55.3 (<u>*C*</u>H), 41.9 (lactam <u>*C*</u>H_2-NH), 28.8 (lactam <u>*C*</u>H_2-CH), 28.5 (<u>*C*</u>H_2-aryl), 20.8 (lactam <u>*C*</u>H_2-CH_2NH) and 15.1 (<u>*C*</u>H_3); ESI *m/*z 100 %, 305.1 (MNa^+) and 56 %, 586.7 (M_2Na^+); HR ESI *m/*z ($C_{13}H_{18}N_2O_3SH^+$ requires 283.1111) found 283.1114.

4.32, 4-^{t}Butylbenzenesulfonyl-(*S*)-3-amino-azepan-2-one

Lactam **2.65** (2.35 g, 9.18 mmol) was dissolved in H_2O (20 mL) and cooled to 0°C. 4-^{t}Buytlbenzenesulfonyl chloride **4.27** (1.92 g, 8.25 mmol) in dichloromethane (40 mL) and triethylamine (3.5 mL, 25 mmol) were added and the reaction was stirred over night. The dichloromethane was removed *in vacuo* and the product was dissolved in ethyl acetate and washed with pH 2 buffer (3 × 20 mL) and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 50:50:0 to 0:80:20) to give compound **4.32** as a white solid (0.67 g, 25 %); *mp* 189-190 º C; $[\alpha]^{23}_{D}$ (c = 0.532, $CHCl_3$) +109.68; υ_{max}/cm^{-1}: 3219 (N-H), 2968 (saturated C-H), 1668 (amide C=O), 1594 (aromatic) and 1361 (SO_2); *Anal.* ($C_{16}H_{24}N_2O_3S$ requires: C; 59.23, H; 7.46, N; 8.63) found: C; 59.46, H; 7.48, N; 8.65; δ_H (400 MHz, $CDCl_3$) 7.74 (d, 2H, *J* 8.5, C*H*-CSO_2), 7.47 (d, 2H, *J* 8.5, C*H*-C^{t}Bu), 6.27 (br.t, 1H, *J* 6.5, N*H*-CH_2), 6.19 (d, 1H, *J* 4.5, N*H*-CH), 3.88-3.81 (m, 1H, C*H*), 3.21-3.12 (m, 1H, lactam C*H*$_2$-NH), 3.05 (ddd, 1H, *J* 14.5, 11.5, 5, lactam C*H*$_2$-NH), 2.19-2.13 (m, 1H, lactam C*H*$_2$-CH), 2.02-1.96 (m, 1H, lactam C*H*$_2$-CH_2NH), 1.81-1.74 (m, 1H, lactam C*H*$_2$-CH_2NH), 1.71-1.54 (m, 2H, lactam C*H*$_2$-CH and lactam C*H*$_2$-CH_2CH), 1.33-1.29 (m, 1H, lactam C*H*$_2$-CH_2CH) and 1.31 (s, 3H, C(C*H*$_3$)$_3$); δ_C (100 MHz, $CDCl_3$) 175.5 (*C*=O), 156.4 (*C*-C$(CH_3)_3$), 137.0 (*C*-CO), 126.8 (*C*H-C^{t}Bu), 126.1 (*C*H-CSO_2), 55.4 (*C*H), 42.3 (lactam *C*H_2-NH), 35.2 (*C*$(CH_3)_3$), 33.5 (lactam *C*H_2-CH), 31.1 (C(*C*$H_3)_3$), 28.7 (lactam *C*H_2-CH_2NH) and 28.0 (lactam *C*H_2-CH_2CH); ESI *m/*z 100 %, 347.1 (MNa^+) and 26 %, 670.6 (M_2Na^+); HR ESI *m/*z ($C_{16}H_{24}N_2O_3SH^+$ requires 325.1580) found 325.1580.

4.33, 4-*tert*-Butylbenzenesulfonyl-(*S*)-3-amino-tetrahydropyridin-2-one

Lactam **2.53** (10 mmol) was dissolved in H_2O (40 mL) and cooled to 0°C. 4-*tert*-Buytlbenzenesulfonyl chloride **4.27** (1.88 g, 8.08 mmol) in dichloromethane (25 mL) and triethylamine (3.5 mL, 25 mmol) were added and was stirred over night. The reaction was extracted with dichloromethane (3 × 20 mL), the organic layer was washed with a pH 2 buffer (3 × 20 mL) and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate:MeOH 50:50:0 to 0:80:20) to give compound **4.33** as a white solid (0.76 g, 30 %); *mp* 155-156 º C; $[\alpha]^{23}_D$ (c = 0.525, $CHCl_3$) +122.92; υ_{max}/cm^{-1}: 3220 (N-H), 2946 (saturated C-H), 1665, 1596 (aromatic) and 1331, 1134 (SO_2); δ_H (400 MHz, $CDCl_3$) 7.79 (d, 2H, *J* 8.5, C*H*-SO_2), 7.47 (d, 2H, *J* 8.5, C*H*-C^{t}Bu), 6.38 (br.s, 1H, N*H*-CH_2), 5.98 (d, 1H, *J* 3.5, N*H*-CH), 3.51-3.46 (m, 1H, C*H*), 3.28-3.22 (m, 2H, lactam C*H*$_2$-NH), 2.49-2.42 (m, 1H, lactam C*H*$_2$-CH), 1.93-1.87 (m, 1H, lactam C*H*$_2$-CH_2NH), 1.86-1.67 (m, 2H, lactam C*H*$_2$-CH_2NH and C*H*$_2$-CH) and 1.31 (s, 9H, C(C*H*$_3$)$_3$); δ_C (100 MHz, $CDCl_3$) 170.1 (*C*=O lactam), 156.5 (*C*-C$(CH_3)_3$), 136.0 (*C*-SO_2), 127.1 (*C*H-C^{t}Bu), 126.2 (*C*H-CSO_2), 53.2 (*C*H), 41.8 (lactam *C*H_2-NH), 35.2 (*C*$(CH_3)_3$), 31.1 (C(*C*H_3)$_3$), 28.4 (lactam *C*H_2-CH) and 20.7 (lactam *C*H_2-CH_2NH); ESI *m/*z 100 %, 333.1 (MNa^+) and 44 %, 642.6 (M_2Na^+); HR ESI *m/*z ($C_{15}H_{22}N_2O_3SH^+$ requires 311.1424) found 311.1425.

4.34, 4-*tert*-Butylbenzenesulfonyl-(*R*)-3-amino-tetrahydropyridin-2-one

Lactam **2.54** (2.5 mmol) was dissolved in H_2O (30 mL) and cooled to 0°C. 4-*tert*-Buytlbenzenesulfonyl chloride **4.27** (0.61 g, 2.6 mmol) in dichloromethane (25 mL)

and triethylamine (1.1 mL, 7.5 mmol) were added and stirred over night. The reaction was extracted with dichloromethane (3 × 20 mL), the organic layer was washed with a pH 2 buffer (3 × 20 mL) and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 75:25 to 0:100) to give compound **4.34** as a white solid (0.33 g, 43 %) *mp* 155-156 °C; $[\alpha]^{25}_{D}$ (c = 0.114, $CHCl_3$) -116.52; υ_{max}/cm^{-1}: 3384, 3226 (N-H), 2927 (saturated C-H), 1663 (amide C=O) and 1330, 1163 (SO_2); δ_H (400 MHz, $CDCl_3$) 7.80 (d, 2H, *J* 8.5, C*H*-SO_2), 7.50 (d, 2H, *J* 8.5, C*H*-C-^{t}Bu), 5.99 (br.s, 1H, N*H*-CH_2), 5.90 (br.d, 1H, *J* 2, N*H*-CH), 3.51-3.46 (m, 1H, C*H*), 3.31-3.26 (m, 2H, lactam C*H*$_2$-NH), 2.53-2.43 (m, 1H, lactam *C*H_2-CH), 1.96-1.89 (m, 1H, lactam *C*H_2-CH_2NH), 1.85-1.69 (m, 2H, lactam *C*H_2-CH_2NH and *C*H_2-CH) and 1.32 (s, 9H, C(C*H*$_3$)$_3$); δ_C (100 MHz, $CDCl_3$) 169.9 (*C*=O), 156.6 (*C*-$C(CH_3)_3$), 135.8 (*C*-SO_2), 127.2 (*C*H-C^{t}Bu), 126.2 (*C*H-CSO_2), 53.3 (*C*H), 42.0 (lactam *C*H_2-NH), 35.2 (*C*$(CH_3)_3$), 31.1 (C(*C*H_3)$_3$), 28.5 (lactam *C*H_2-CH) and 20.8 (lactam *C*H_2-CH_2NH); ESI *m/*z 37 %, 642.6 (M_2Na^+); HR ESI *m/*z ($C_{15}H_{22}N_2O_3SH^+$ requires 311.1424) found 311.1427.

4.35, 4-Octylbenzenesulfonyl-(*S*)-3-amino-azepan-2-one

Lactam **2.65** (0.73 g, 4.5 mmol) was dissolved in H_2O (30 mL) and cooled to 0°C. Sulfonyl chloride **4.28** (2.2 mmol) in dichloromethane (25 mL) and triethylamine (0.90 mL, 6.6 mmol) were added and stirred over night. The reaction was extracted with dichloromethane (3 × 20 mL) the organic layer was washed with a pH 2 buffer (3 × 20 mL) and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 50:5 to 0:100) to give compound **4.35** as a white solid (0.49 g, 59 %); *mp* 98-99 º C; $[\alpha]^{30}_{D}$ (c = 0.26, $CHCl_3$) +91.15; υ_{max}/cm^{-1}: 3396, 3250 (N-H), 2920 (saturated C-H), 1663 (amide C=O) and 1363,

1161 (SO_2); *Anal.* ($C_{21}H_{34}N_2O_2$ requires: C; 73.22, H; 9.36, N; 8.13) found: C; 72.5, H; 9.35, N; 9.98; δ_H (400 MHz, $CDCl_3$) 7.71 (d, 2H, *J* 8.5, C*H*-CSO_2), 7.25 (d, 2H, *J* 8.5, C*H*-COct), 6.68 (dd, 1H, *J* 7.5, 5,5 N*H*-CH_2), 6.22 (d, 1H, *J* 5, N*H*-CH), 3.81 (ddd, 1H, *J* 2, 5, 11 C*H*), 3.20-3.311 (m, 1H, lactam C*H*$_2$-NH), 3.02 (ddd, 1H, *J* 16, 11.5, 5, lactam C*H*$_2$-NH), 2.63 (t, 2H, *J* 8, C*H*$_2$-aryl), 2.12-2.07 (m, 1H, lactam C*H*$_2$-CH), 1.98-1.93 (m, 1H, lactam C*H*$_2$-CH_2CH), 1.79-1.72 (m, 1H, lactam C*H*$_2$-CH_2NH), 1.65-1.55 (m, 4H, lactam C*H*$_2$-CH, C*H*$_2$-CH_2CH and lactam C*H*$_2$-aryl), 1.33-1.21 (m, 11H, lactam C*H*$_2$-CH_2NH and C*H*$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$-CH_3) and 0.86 (t, 3H, *J* 7, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 174.6 (*C*=O), 148.4 (*C*-Oct), 137.2 (*C*-SO_2), 129.1 (*C*H-COct), 128.8 (*C*H-CSO_2), 127.0 (phenyl *C*H), 55.3 (*C*H), 42.1 (lactam *C*H_2-NH), 35.8 (lactam *C*H_2-CH), 33.3 (*C*H_2-aryl), 31.8 (lactam *C*H_2-CH_2CH), 31.0 (*C*H_2-CH_2-aryl), 29.4 (*C*H_2-CH_2CH_2-aryl), 29.2 (*C*H_2-$CH_2CH_2CH_2$-aryl), 28.6 (*C*H_2-$CH_2CH_2CH_3$), 28.0 (*C*H_2-CH_2CH_3), 22.7 (lactam *C*H_2-CH_2NH), 22.6 (*C*H_2-CH_3) and 14.1 (*C*H_3); ESI *m/*z 100 %, 403.2 (MNa^+) and 40 %, 381.2 (MH^+); HR ESI *m/*z ($C_{20}H_{32}N_2O_3SH^+$ requires 381.2206) found 381.2205.

4.36, 4-Octylbenzenesulfonyl-(*S*)-3-amino-tetrahydropyridin-2-one

Lactam **2.53** (2.5 mmol) was dissolved in H_2O (40 mL) and cooled to 0°C. Sulfonyl chloride **4.28** (1.34 mmol) in dichloromethane (25 mL) and triethylamine (0.6 mL, 4 mmol) were added and stirred over night. The reaction was extracted with dichloromethane (3 × 20 mL) the organic layer was washed with a pH 2 buffer (3 × 20 mL) and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate:MeOH 50:50:0 to 0:80:20) to give compound **4.36** as a white solid (0.31 g, 63 %); *mp* 98-99 ° C; $[\alpha]^{24}_D$ (c = 0.515, $CHCl_3$) +99.97; υ_{max}/cm^{-1}: 3207 (N-H), 2920 (saturated C-H), 1664, (C=O), 1544

(aromatic) and 1310, 1188 (SO_2); *Anal.* ($C_{10}H_{18}N_2O_2$ requires: C; 62.26, H; 8.25, N; 7.64) found: C; 62.30, H; 8.26, N; 7.65; δ_H (400 MHz, $CDCl_3$) 7.76 (d, 2H, *J* , C*H*-CSO_2), 7.27 (d, 2H, *J* 8, C*H*-C-Oct), 6.50 (br.s, 1H, N*H*-CH_2), 6.00 (d, 1H, *J* 2.5, N*H*-CH), 3.51-3.45 (m, 1H, lactam C*H*), 3.27-3.31 (m, 2H, lactam C*H*$_2$-NH), 2.62 (t, 2H, *J* 7, C*H*$_2$-aryl), 2.45-2.36 (m, 1H, lactam C*H*$_2$-CH), 1.92-1.85 (m, 1H, lactam C*H*$_2$-CH_2NH), 1.80-1.67 (m, 2H, lactam C*H*$_2$-CH_2NH and lactam C*H*$_2$-CH), 1.62-1.55 (m, 2H, C*H*$_2$-CH_2-aryl), 1.31-1.20 (m, 10H, C*H*$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$-CH_3) and 0.85 (t, 3H, *J* 7, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 170.1 (*C*=O), 148.5 (*C*-Oct), 136.3 (*C*-SO_2), 129.1 (*C*H-COct), 127.3 (*C*H-CSO_2), 53.2 (*C*H-NH), 41.8 (lactam *C*H_2-NH), 35.9 (*C*H_2-aryl), 31.8 (*C*H_2-CH_2-aryl), 31.0 (lactam *C*H_2-CH), 29.4 (*C*H_2-CH_2-CH_2-aryl), 29.3 (*C*H_2-$CH_2CH_2CH_2CH_3$), 29.2 (*C*H_2-$CH_2CH_2CH_2CH_3$), 28.4 (*C*H_2-CH_2CH_3), 22.7 (*C*H_2-CH_3), 20.7 (lactam *C*H_2-CH_2NH) and 14.1 (*C*H_3); ESI *m/*z 100 %, 389.2 (MNa^+) and 36 %, 367.2 (MH^+); HR ESI *m/*z ($C_{19}H_{30}N_2O_3SNa^+$ requires 389.1869) found 389.1865.

4.37, 4-Octylbenzenesulfonyl-(*R*)-3-amino-tetrahydropyridin-2-one

Lactam **2.54** (2.5 mmol) was dissolved in H_2O (30 mL) and cooled to 0°C. Sulfonyl chloride **4.28** (1.34 mmol) in dichloromethane (25 mL) and triethylamine (0.57 mL, 4.0 mmol) were added and stirred over night. The reaction was extracted with dichloromethane (3 × 20 mL) the organic layer was washed with a pH 2 buffer (3 × 20 mL) and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 50:50 to 0:100) to give compound **4.37** as a white solid (0.22 g, 45 %); *mp* 98-99 ° C; $[\alpha]^{25}_D$ (c = 0.238, $CHCl_3$) +102.94; υ_{max}/cm^{-1}: 3226 (N-H), 2920 (saturated C-H), 1663 (amide C=O) and 1328, 1160 (SO_2); δ_H (400 MHz, $CDCl_3$) 7.76 (d, 2H, *J* 8, C*H*-CSO_2), 7.26 (d, 2H, *J*

8, C*H*-C-Oct), 6.85 (br.s, 1H, N*H*-CH_2), 6.14 (d, 1H, *J* 3, N*H*-CH), 3.52-3.46 (m, 1H, lactam C*H*), 3.23-3.16 (m, 2H, lactam C*H*$_2$-NH), 2.62 (t, 2H, *J* 7, C*H*$_2$-aryl), 2.38-2.31 (m, 1H, lactam C*H*$_2$-CH), 1.87-1.81 (m, 1H, lactam C*H*$_2$-CH_2NH), 1.74-1.65 (m, 2H, lactam C*H*$_2$-CH_2NH and lactam C*H*$_2$-CH), 1.62-1.54 (m, 2H, C*H*$_2$-CH_2-aryl), 1.32-1.20 (m, 10H, C*H*$_2$C*H*$_2$C*H*$_2$C*H*$_2$C*H*$_2$$CH_3$) and 0.84 (t, 3H, *J* 7, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 170.2 (*C*=O), 148.4 (*C*-Oct), 136.5 (*C*-SO_2), 129.0 (*C*H-COct), 127.3 (*C*H-CSO_2), 53.2 (*C*H-NH), 41.5 (lactam *C*H_2-NH), 35.8 (*C*H_2-aryl), 32.0 (*C*H_2-CH_2-aryl), 31.8 (lactam *C*H_2-CH), 29.4 (*C*H_2-CH_2-CH_2-aryl), 29.3 (*C*H_2-$CH_2CH_2CH_2CH_3$), 29.1 (*C*H_2-$CH_2CH_2CH_2CH_3$), 25.5 (*C*H_2-$CH_2CH_2CH_3$), 22.7 (*C*H_2-CH_3), 20.7 (lactam *C*H_2-CH_2NH) and 14.1 (*C*H_3); ESI *m/*z 15 %, 389.2 (MNa^+); HR ESI *m/*z ($C_{19}H_{30}N_2O_3SNa^+$ requires 389.1869) found 389.1872.

4.38, 4-Chlorobenzene isopropylsulfonate

4-(Dimethylamino) pyridine (3.75 g, 30.1 mmol) was dissolved in dichloromethane (40 mL) and propan-2-ol (2 mL, 30 mmol) and cooled to 0 °C. A solution of 4-chlorobenzenesulfonyl chloride (1.63 g, 7.7 mmol) in dichloromethane (10 mL) was added drop wise and the reaction was allowed to stir at room temperature for 3 hours. The reaction was washed with 1 M HCl (3 × 15mL) and NaCl (3 × 15mL), dried over Na_2SO_4 and reduced *in vacuo* to give compound **4.38** as a colourless oil 1.64 g (64 %); υ_{max}/cm^{-1}: 2984 (saturated C-H), 1583 (aromatic), 1362, 1184 (SO_2) and 779 (C-Cl); δ_H (400 MHz, $CDCl_3$) 7.77 (d, 2H, *J* 8.5, C*H*-CSO_2), 7.44 (d, 1H, *J* 8.5, C*H*-C-Cl), 4.69 (septet, 1H, *J* 6.5, C*H*-O) and 1.21 (d, 6H, *J* 6.5, (C*H*$_3$)$_2$); δ_C (100 MHz, $CDCl_3$) 140.0 (*C*-Cl), 136.0 (*C*-SO_2), 129.3 (*C*H-C-SO_2), 129.0 (*C*H-C-Cl), 78.1 (*C*H-O) and 22.8 (*C*H_3)$_2$; ESI *m/*z 100 %, 257.0 (MNa^+), 54 %, 490.7

(M_2Na^+) and 32 %, 215.9 (M-(C_3H_6)Na^+). Data is consistent with previously reported data for this compound.[246]

4.39, 4-^{n}Butylbenzene isopropylsulfonate

Compound **4.38** (7 mmol) was dissolved in THF (25 mL) and cooled to 0 °C. $Fe(acac)_3$ (0.136 g, 0.39 mmol) and NMP (4 mL, 56 mmol) were added. The reaction was stirred under nitrogen gas and BuMgCl (4.2 mL, 2M in THF) was added drop wise with a syringe. The reaction was stirred for 5 minutes before quenching with pH 2 buffer and extracting with ethyl acetate (3 × 15mL) and washing with pH 2 buffer (3 × 15mL). The product was purified by silica column chromatography (petroleum ether:ethyl acetate 75:25) to give compound **4.39** as a colourless oil 1.02 g (57 %); υ_{max}/cm^{-1}: 2932 (saturated C-H), 1598 (aromatic) and 1348, 1173 (SO_2); δ_H (400 MHz, $CDCl_3$) 7.76 (d, 2H, *J* 8.5, C*H*-CSO_2), 7.29 (d, 1H, *J* 8.5, C*H*-CBu), 4.68 (septet, 1H, *J* 6, C*H*-O), 2.64 (t, 2H, *J* 8, C*H*$_2$-aryl), 1.57 (quin., 2H, *J* 8, C*H*$_2$-CH_2-aryl), 1.30 (sextet, 2H, *J* 8, C*H*$_2$-CH_3), 1.21 (d, 6H, *J* 6, (C*H*$_3$)$_2$) and 0.87 (t, 3H, *J* 8, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 149.3 (*C*-Bu), 134.6 (*C*-SO_2), 128.9 (*C*H-CBu), 127.5 (*C*H-CSO_2), 77.0 (*C*H-O), 35.7 (*C*H_2-aryl), 33.1 (*C*H_2-CH_2-aryl), 22.7 (*C*H_3)$_2$, 22.2 (*C*H_2-CH_3) and 13.8 (*C*H_3); ESI *m/*z 100 %, 534.7 (M_2Na^+) and 23 %, 279.1 (MNa^+).

4.40, 4-^{n}Hexylbenzene isopropylsulfonate

Compound **4.38** (2.46 g, 10.5 mmol) was dissolved in THF (25 mL) and cooled to 0 °C. $Fe(acac)_3$ (0.22 g, 0.525 mmol) and NMP (6 mL, 63 mmol) were added. The reaction was stirred under nitrogen gas and HexMgCl (6.6 mL, 2M in THF) was added drop wise with a syringe. The reaction was stirred for 5 minutes before

quenching with pH 2 buffer and extracting with ethyl acetate (3 × 15mL) and washing with pH 2 buffer (3 × 15mL). The product was purified by silica column chromatography (petroleum ether:ethyl acetate 75:25) to give compound **4.40** as a colourless oil 1.30 g (44 %); δ_H (400 MHz, $CDCl_3$) 7.78 (d, 2H, *J* 8, C*H*-CSO_2), 7.30 (d, 1H, *J* 8, C*H*-CBu), 4.70 (septet, 1H, *J* 6, C*H*-O), 2.65 (t, 2H, *J* 8, C*H*$_2$-aryl), 1.60 (quin., 2H, *J* 7.5, C*H*$_2$-CH_2-aryl), 1.33-1.22 (m, 12H, C*H*$_2$-C*H*$_2$-C*H*$_2$-CH_3 and (C*H*$_3$)$_2$)) and 0.84 (t, 3H, *J* 7, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 149.3 (*C*-Hex), 134.7 (*C*-SO_2), 128.8 (*C*H-CHex), 127.7 (*C*H-CSO_2), 77.1 (*C*H-O), 35.9 (*C*H_2-aryl), 33.0 (*C*H_2-CH_2-aryl), 29.9 (*C*H_2-CH_2CH_2-aryl), 28.6 (*C*H_2-CH_2CH_3), 22.8 (*C*H_3)$_2$, 22.7 (*C*H_2-CH_3) and 14.2 (*C*H_3); ESI *m/*z 100 %, 590.6 (M_2Na^+) and 73 %, 307.1 (MNa^+).

4.41, 4-(Butylbenzenesodiumsulfonate)

Sulphanate ester **4.39** (7 mmol) and NaI (5.2 g, 34.69 mmol) were dissolved in acetone (25 mL) and stirred under nitrogen and in the absence of light under reflux conditions for 16 hours. The acetone was removed *in vacuo* and the product was washed with ethyl acetate (3 × 25 mL) and acetone (3 × 25 mL) to give compound **4.41** as a white solid, the product still contains NaI so the yield was not calculated; δ_H (400 MHz, $CDCl_3$) 7.49 (d, 2H, *J* 8, C*H*-CSO_2), 7.12 (d, 1H, *J* 8, C*H*-CBu), 2.56 (t, 2H, *J* 8, C*H*$_2$-aryl), 1.53 (quin., 2H, *J* 8, C*H*$_2$-CH_2-aryl), 1.29 (sextet, 2H, *J* 7.5, C*H*$_2$-CH_3) and 0.89 (t, 3H, *J* 7.5, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 149.3 (*C*-Bu), 134.6 (*C*-SO_2), 128.9 (*C*H-CBu), 127.5 (*C*H-CSO_2), 77.0 (*C*H-O), 35.7 (*C*H_2-aryl), 33.1 (*C*H_2-CH_2-aryl), 22.7 (*C*H_3)$_2$, 22.2 (*C*H_2-CH_3) and 13.8 (*C*H_3).

4.42, 4-(Hexylbenzenesodiumsulfonate)

Sulfonate ester **4.40** (1.30 g, 4.57 mmol) and NaI (8 g, 53.37 mmol) were dissolved in acetone (20 mL) and stirred under nitrogen and in the absence of light under reflux conditions for 16 hours. The acetone was removed *in vacuo* and the product was washed with ethyl acetate (3 × 25 mL) and acetone (3 × 25 mL) to give compound **4.42**. The product still contains NaI so the yield was not calculated; δ_H (400 MHz, $CDCl_3$) 7.50 (d, 2H, *J* 8.5, C*H*-CSO_2), 7.13 (d, 1H, *J* 8.5, C*H*-C-Bu), 2.55 (t, 2H, *J* 7.5, C1), 1.54 (quintet, 2H, *J* 7.5, H2), 1.28-1.22 (m, 6H, *J* 7.5, H3, H4 & H5) and 0.84 (t, 3H, *J* 7.5, H6); δ_C (100 MHz, $CDCl_3$) 145.6 (*C*-Hex), 142.7 (*C*-SO_2ONa), 127.5 (*C*H-CHex), 125.5 (*C*H-CSO_2), 34.7 (*C*H_2-aryl), 31.1 (*C*H_2-CH_2-aryl), 30.8 (*C*H_2-CH_2CH_2-aryl), 28.2 (*C*H_2-CH_2CH_3), 22.0 (*C*H_2-CH_3) and 14.2 (*C*H_3).

4.43, 4-(Butylbenzenesulfonylchloride)

Sodium salt **4.41** (7 mmol) was dissolved in pH 2 buffer and extracted with ethyl acetate (3 × 15 mL). The organic layer was dried over Na_2SO_3 and reduced *in vacuo* to give the corresponding sulfonic acid, which was dissolved in dichloromethane. Oxalyl chloride (0.50 mL, 7.7 mmol) and DMF (1 drop) were added and the reaction stirred for 3 hours. The organic solvents were removed *in vacuo* to give compound **4.43** which was not isolated and used in the next step.

4.44, 4-(Hexylbenzenesulfonylchloride)

Sodium salt **4.24** (4.57 mmol) was dissolved in pH 2 buffer and extracted with ethyl acetate (3 × 15 mL). The organic layer was dried over Na_2SO_3 and reduced *in vacuo* to give the corresponding sulfonic acid, which was dissolved in dichloromethane.

Oxalyl chloride (0.33 mL, 5.03 mmol) and DMF (1 drop) were added and the reaction stirred for 3 hours. The organic solvents were removed *in vacuo* to give compound **4.44** which was not isolated and used in the next step.

4.45, 4-Butylbenzenesulfonyl-(*S*)-3-amino-azepan-2-one

Lactam **2.65** (1.15 g, 7.01 mmol) was dissolved in H_2O (20 mL) and cooled to 0°C. Sulfony chloride **4.43** (7 mmol) in dichloromethane (30 mL) and triethylamine (3 mL, 21 mmol) were added and stirred over night. The reaction was extracted with dichloromethane and washed with pH 2 buffer (3 × 20 mL) and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 75:25 to 0:100) to give compound **4.45** as a white solid (0.17 g, 19 %); *mp* 155-156 ° C; $[\alpha]^{28}_{D}$ (c = 0.11, $CHCl_3$) +94.39; υ_{max}/cm^{-1}: 3219 (N-H), 2914 (saturated C-H), 1668 (amide C=O), 1596 (aromatic) and 1328, 1121 (SO_2); δ_H (400 MHz, $CDCl_3$) 7.76 (d, 2H, *J* 8, C*H*-CSO_2), 7.31 (d, 2H, *J* 7.5, C*H*-C^{n}Bu), 6.76 (br.t, 1H, *J* 6, N*H*-CH_2), 6.28 (d, 1H, *J* 5, N*H*-CH), 3.85 (ddd, 1H, *J* 11, 5, 2, C*H*), 3.24-3.16 (m, 1H, lactam C*H*$_2$-NH), 3.06 (ddd, 1H, *J* 15, 12, 5, lactam C*H*$_2$-NH), 2.68 (t, 2H, *J* 8, C*H*$_2$-aryl), 2.18-2.11 (m, 1H, lactam C*H*$_2$-CH), 2.03-1.93 (m, 1H, lactam C*H*$_2$-CH_2CH), 1.84-1.73 (m, 1H, lactam C*H*$_2$-CH_2NH), 1.69-1.55 (m, 4H, lactam C*H*$_2$-CH_2CH, C*H*$_2$-CH and C*H*$_2$-CH_2-aryl), 1.38 (sextet, 2H, *J* 7.5, C*H*$_2$-CH_3), 1.36-1.25 (m, 1H, lactam C*H*$_2$-CH_2NH) and 0.95 (t, 3H, *J* 7.5, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 175.6 (*C*=O), 148.3 (*C*-Bu), 137.1 (*C*-SO_2), 129.1 (*C*H-C^{n}Bu), 127.0 (*C*H-CSO_2), 55.3 (*C*H), 42.1 (lactam *C*H_2-NH), 35.5 (lactam *C*H_2-CH), 33.3 (*C*H_2-aryl), 33.1 (lactam *C*H_2-CH_2CH), 28.6 (*C*H_2-CH_2-aryl), 28.0 (*C*H_2-CH_3), 22.3 (lactam *C*H_2-CH_2NH) and 13.9 (*C*H_3); ESI *m/*z 100 %, 670.6 (M_2Na^+), 86 %, 347.1 (MNa^+)

and 43 %, 325.1 (MH^+); HR ESI *m/*z ($C_{16}H_{24}N_2O_3SNa^+$ requires 347.1400) found 347.1397.

4.46, 4-Butylbenzenesulfonyl-(*S*)-3-amino-tetrahydropyridin-2-one

Lactam **2.53** (0.5 mmol) was dissolved in H_2O (2 mL) and cooled to 0°C. Sulfonyl chloride **4.43** (0.1 mmol) in dichloromethane (3 mL) and triethylamine (42 µL, 0.30 mmol) were added and stirred over night. The reaction was extracted with dichloromethane (3 × 5 mL) and washed with pH 2 buffer (3 × 5 mL) and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 50:50 to 0:100) to give compound **4.46** as a white solid (0.0.15 g, 48 %); *mp* 107-108 º C; $[\alpha]^{28}_D$ (c = 0.44, $CHCl_3$) +109.70; υ_{max}/cm^{-1}: 3192 (N-H), 2928 (saturated C-H), 1662 (C=O), 1597 (aromatic) and 1312, 1160 (SO_2); δ_H (400 MHz, $CDCl_3$) 7.76 (d, 2H, *J* 8, C*H*-C-SO_2), 7.27 (d, 2H, *J* 8, C*H*-C^{n}Bu), 6.45 (br.s, 1H, N*H*-C1), 5.98 (d, 1H, *J* 2.5, N*H*-CH), 3.51-3.44 (m, 1H, C*H*-C3), 3.28-3.20 (m, 2H, H1), 2.63 (t, 2H, *J* 8, H4), 2.44-2.38 (m, 1H, H3), 1.92-1.83 (m, 1H, H2), 1.81-1.66 (m, 2H, H2 and H3), 1.58 (quartet, 2H, *J* 7.5, H5), 1.33 (sextet, 2H, H6) and 0.95 (t, 3H, *J* 7.5, H7); δ_C (100 MHz, $CDCl_3$) 170.1 (*C*=O), 148.5 (*C*-Bu), 136.3 (*C*-SO_2), 129.1 (*C*H-C^{n}Bu), 127.3 (*C*H-CSO_2), 53.2 (*C*H-NH), 41.8 (C1), 35.6 (C3), 33.1 (C4), 28.4 (C5), 22.3 (C6), 20.7 (C2) and 13.9 (C7); ESI *m/*z 100 %, 642.6 (M_2Na^+) and 71 %, 333.1 (MNa^+); HR ESI *m/*z ($C_{15}H_{22}N_2O_3SNa^+$ requires 333.1243) found 333.1247.

4.47, 4-Hexylbenzenesulfonyl-(*S*)-3-amino-azepan-2-one

Lactam **2.65** (1.03 g, 4.00 mmol) was dissolved in H_2O (10 mL) and cooled to 0°C. Sulfonyl chloride **4.44** (3 mmol) in dichloromethane (10 mL) and triethylamine (1.3

mL, 9.0 mmol) were added and the reaction was stirred over night. The reaction was extracted with dichloromethane and washed with pH 2 buffer (3 × 20 mL) and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 75:25 to 0:100) to give compound **4.47** as a white solid 0.49 g (46 %); *mp* 115-116 ° C; $[\alpha]^{28}_{D}$ (c = 0.227, $CHCl_3$) +104.41; υ_{max}/cm^{-1}: 3371, 3242 (N-H), 2928 (saturated C-H), 1661 (C=O), 1599 (aromatic) and 1329, 1161 (SO_2); *Anal.* ($C_{18}H_{28}N_2O_3S$ requires: C; 61.33, H; 8.01, N; 7.95) found: C; 61.21, H; 8.04, N; 7.97; δ_H (400 MHz, $CDCl_3$) 7.66 (d, 2H, *J* 8.5, C*H*-C-SO_2), 7.20 (d, 2H, *J* 8.5, C*H*-CHex), 7.06 (br.t, 1H, *J* 6, N*H*-CH_2), 6.29 (d, 1H, *J* 5, N*H*-CH), 3.75 (br.d, 1H, *J* 11, lactam C*H*), 3.14-3.97 (m, 1H, lactam C*H*$_2$-NH), 2.98-2.88 (m, 1H, lactam C*H*$_2$-NH), 2.57 (t, 2H, *J* 8, C*H*$_2$-aryl), 2.00 (br.d, 1H, *J* 13, lactam C*H*$_2$-CH), 1.87 (br.d, 1H, *J* 13, lactam C*H*$_2$-CH_2CH), 1.68 (br.d, 1H, *J* 13.5, lactam C*H*$_2$-CH_2NH), 1.61-1.45 (m, 3H, lactam C*H*$_2$-CH and C*H*$_2$-CH_2-aryl), 1.29-1.17 (m, 8H, lactam C*H*$_2$-CH_2NH, lactam C*H*$_2$-CH_2CH and C*H*$_2$-C*H*$_2$-C*H*$_2$-CH_3) and 0.82-0.77 (m, 3H, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 174.7 (*C*=O), 148.3 (*C*-Hex), 137.2 (*C*-SO_2), 129.0 (*C*H-CHex), 126.9 (*C*H-CSO_2), 55.3 (*C*H-NH), 42.0 (lactam *C*H_2-NH), 35.8 (lactam *C*H_2-CH), 33.2 (*C*H_2-aryl), 31.5 (lactam *C*H_2-CH_2CH), 30.9 (*C*H_2-CH_2-aryl), 28.8 (*C*H_2-CH_2CH_2-aryl), 28.5 (*C*H_2-CH_2CH_3), 27.9 (lactam *C*H_2-CH_2NH), 22.5 *C*H_2-CH_3) and 14.0 (*C*H_3); ESI *m/*z 100 %, 375.1 (MNa^+) and 15 %, 353.1 (MH^+); HR ESI *m/*z ($C_{18}H_{28}N_2O_3SH^+$ requires 353.1893) found 353.1896.

4.48, 4-Hexylbenzenesulfonyl-(*S*)-3-tetrahydropyridin-2-one

Lactam **2.53** (1 mmol) was dissolved in H_2O (5 mL) and cooled to 0°C. Sulfonyl chloride (0.25 mmol) in dichloromethane (5 mL) and triethylamine (0.10 mL, 0.75 mmol) were added and stirred over night. The reaction was extracted with

dichloromethane and washed with pH 2 buffer (3 × 5 mL) and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 75:25 to 0:100) to give compound **4.48** as a white solid (0.042 g, 17 %); *mp* 97-98 ^{o}C; $[\alpha]^{30}_{D}$ (c = 0.508, $CHCl_3$) +106.77; υ_{max}/cm^{-1}: 3209 (N-H), 2925 (saturated C-H), 1662 (C=O), 1595 (aromatic) and 1326, 1107 (SO_2); δ_H (400 MHz, $CDCl_3$) 7.77 (d, 2H, *J* 8, C*H*-CSO_2), 7.29 (d, 2H, *J* 8, C*H*-CHex), 6.14 (br.s, 1H, N*H*-CH_2), 5.91 (br.s, 1H, N*H*-CH), 3.51-3.45 (m, 1H, lactam C*H*), 3.31-3.22 (m, 2H, lactam C*H*$_2$-NH), 2.64 (t, 2H, *J* 7.5, C*H*$_2$-aryl), 2.49-2.42 (m, 1H, lactam C*H*$_2$-CH), 1.94-1.87 (m, 1H, lactam C*H*$_2$- CH_2NH), 1.82-1.69 (m, 2H, lactam C*H*$_2$-CH and lactam C*H*$_2$-CH_2NH), 1.60 (q, 2H, *J* 7.5, C*H*$_2$-CH_2-aryl), 1.35-1.25 (m, 6H, C*H*$_2$-C*H*$_2$-C*H*$_2$-CH_3) and 0.86 (dd, 3H, *J* 6.5, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 170.0 (*C*=O), 148.6 (*C*-Hex), 136.2 (*C*-SO_2), 129.1 (*C*H-CHex), 127.4 (*C*H-CSO_2), 53.3 (*C*H-NH), 41.9 (lactam *C*H_2-NH), 35.9 (lactam *C*H_2-CH), 31.6 (*C*H_2-aryl), 31.0 (*C*H_2-CH_2-aryl), 28.9 (*C*H_2-CH_2CH_2-aryl), 28.5 (*C*H_2-CH_2CH_3), 22.6 (*C*H_2-CH_3), 20.7 (lactam *C*H_2-CH_2NH), and 14.1 (*C*H_3); ESI *m/*z 100 %, 698.6 (M_2Na^+) and 80 %, 361.1 (MNa^+); HR ESI *m/*z ($C_{17}H_{26}N_2O_3SNa^+$ requires 361.1556) found 361.1564.

6.4.3 2- and 3-Carboxy Compounds

4.49, Methyl-*meta*-chloro-benzoate

Acetyl chloride (1.3 mL, 15 mmol) was dissolved in MeOH (25 mL) and 3-chlorobenzoic acid (1.95 g, 12.5 mmol) was added, the reaction was heated under reflux conditions over night then reduced *in vacuo* to give compound **4.49** as a colourless oil, ^{1}H NMR showed 100 % conversion; δ_H (400 MHz, $CDCl_3$) 7.92 (s, 1H, C-C*H*-C), 7.83 (d, 1H, *J* 8, C*H*-CCOO), 7.43 (d, 1H, *J* 8.5, C*H*-CCl), 7.29 (t, 1H, *J* 8, C*H*-CH-CCl) and 3.85 (s, 3H, OC*H*$_3$); δ_C (100 MHz, $CDCl_3$) 167.7 (C=O),

134.4 (*C*-Cl), 132.9 (*C*H-CCl), 131.8 (*C*-COO), 129.9, 129.8 (C-*C*H-C and *C*H-CH-CCl), 127.9 (*C*H-COO) and 52.3 (CH). Data is consistent with data previously reported for this compound.[247]

4.50, Methyl-*ortho*-chloro-benzoate

Acetylchloride (4.0 mL, 56 mmol) was dissolved in MeOH (60 mL) and 2-chlorobenzoic acid (2.49 g, 15.9 mmol) was added, the reaction heated under reflux conditions over night then reduced *in vacuo* to give compound **4.50** as a white solid (2.68 g, 99 %); δ_H (400 MHz, $CDCl_3$) 7.76 (dd, 1H, *J* 7.5, 2, C*H*-CCl), 7.39-7.31 (m, 2H, C*H*-CCO and C*H*-CH-CCl), 7.23 (td, 1H, *J* 7.5, 2, C*H*-CH-CH-CCl), and 3.87 (s, 3H, OCH_3). Data is consistent with data previoulsy reported for this compound.[248]

4.51, Methyl-3-^{n}butylbenzoate

Compound **4.49** (12.5 mmol) was dissolved in THF (30 mL) and cooled to 0 °C. $Fe(acac)_3$ (0.23 g, 0.65 mmol) and NMP (7.23 mL, 75 mmol) were added. The reaction was stirred under nitrogen gas and BuMgCl (6.3 mL, 2M in THF) was added drop wise. The reaction was stirred for 15 minutes before quenching with pH 2 buffer (25 mL), extracting with ethyl acetate (3 × 15mL) and washing with pH 2 buffer (3 × 15mL). The NMP was removed by silica column chromatography (petroleum ether:ethyl acetate 75:25) to give compound **4.51** as a colourless oil which also contained some unreacted starting material.

4.52, Methyl-3-hexylbenzoate

Compound **4.49** (3.86 g, 24.7 mmol) was dissolved in THF with $Fe(acac)_3$ (0.88 g, 2.8 mmol) and NMP (14.3 mL, 148 mmol) were added. The reaction was stirred under nitrogen and HexMgCl (15.4 mL, 2M) was added drop wise. The reaction was

stirred for 15 minutes before quenching with pH 2 buffer (35 mL), extracting with ethyl acetate (3 × 15mL) and washing with pH 2 buffer (3 × 15mL). The NMP was removed by silica column chromatography (petroleum ether:ethyl acetate 75:25) and the unreacted started material was removed by hydrolysing the methyl-*meta*-chloro-benzoate ester and extracting out the product with ethyl acetate to give compound **4.52** as a colourless oil (0.64 g, 12 %); δ_H (400 MHz, $CDCl_3$) 7.87-7.82 (m, 2H, CH), 7.36-7.28 (m, 2H, CH), 3.88 (s, 3H, OC$\underline{H}_3$), 2.63 (t, 2H, *J* 8, C$\underline{H}_3$-aryl), 1.61 (quintet, 2H, *J* 7.5, C$\underline{H}_2$-CH_2-aryl), 1.36-1.24 (m, 6H, C$\underline{H}_2$-C$\underline{H}_2$-C$\underline{H}_2$-CH_3) and 0.87 (dd, 3H, *J* 8.5, 7.5, C$\underline{H}_3$); δ_C (100 MHz, $CDCl_3$) 167.3 (C=O), 143.2 (*C*-Hex), 133.1 (*C*H-CCO), 130.1 (*C*-CO), 129.5 (*C*H-CCO), 128.3, 127.0 (*C*H-*C*H-CHex), 31.4 (*C*H_2-CH_2CH_2-aryl), 28.9 (*C*H_2-CH_2CH_3), 23.4 (*C*H_2-CH_3) and 14.1 (*C*H_3); ESI *m/*z 100 %, 243.1 (MNa^+) and 38 %, 462.8 (M_2Na^+). Data is consistent with previously reported data for this compound.[249]

4.53, Methyl-2-Butylbenzoate

Compound **4.50** (2.68 g, 15.7 mmol) was dissolved in THF (40 mL) with $Fe(acac)_3$ (0.56 g, 1.6 mmol) and NMP (9.0 mL, 94 mmol). The reaction was stirred under nitrogen and BuMgCl (11.8 mL, 2 M) was added drop wise. The reaction was stirred for 15 minutes before quenching with pH 2 buffer (25 mL), extracted with ethyl acetate (3 × 15mL) and washed with pH 2 buffer (3 × 15mL). The NMP was removed by silica column chromatography (petroleum ether:ethyl acetate 75:25) to give the product compound **4.53** containing some unreacted starting material, the compound was reacted on and purified in the final step.

4.54, 3-Butylbenzoic

Ester **4.51** was dissolved in H_2O and NaOH was added, the reaction was heated over night at 60 °C. The reaction was extracted with ethyl acetate the acidified with pH 2 buffer and extracted again with ethyl acetate, this layer was dried over Na_2SO_3 and reduced *in vacuo* to give compound **4.54** as a colourless oil.

4.55, 3-Hexylbenzoic Acid

Ester **4.52** (0.62 g, 2.8 mmol) was dissolved in H_2O with NaOH (0.56 g, 14 mmol) the reaction was stirred overnight at 50 °C. The reaction was brought to pH 7, extracted with ethyl acetate and reduced *in vacuo* to give compound **4.55** as a white oily solid (0.56 g, 97 %); δ_H (400 MHz, MeOD) 7.83 (br.s, 1H, C*H*-COOH), 7.81-7.79 (CH), 7.31-7.29 (m, 2H, CH), 2.68 (dd, 2H, *J* 8, 7.5, C*H*$_3$-aryl), 1.68 (br.quintet, 2H, *J* 7.5, C*H*$_2$-CH$_2$-aryl), 1.41-1.32 (m, 6H, C*H*$_2$-C*H*$_2$-C*H*$_2$-CH$_3$) and 0.95-0.91 (m, 3H, C*H*$_3$); δ_C (100 MHz, MeOD) 174.7 (C=O), 143.7 (*C*-Hex), 137.4 (*C*-CO), 132.0 (*C*H-CCOOH), 130.4 (*C*H-CCOOH), 128.8, 127.8 (*C*H-*C*H-CHex), 36.9 (*C*H$_2$-aryl), 32.9 (*C*H$_2$-CH$_2$-aryl), 32.7 (*C*H$_2$-CH$_2$CH$_2$CH$_2$CH$_3$), 30.1 (*C*H$_2$-CH$_2$CH$_3$), 23.7 (*C*H$_2$-CH$_3$) and 14.5 (*C*H$_3$); ESI *m/*z 46 %, 229.3 (MNa^+).

4.56, 2-Butylbenzoic acid

Ester **4.53** containing some unreacted starting material was dissolved in H_2O (10 mL) and NaOH (0.24 g, 6 mmoles) was added, the reaction was stirred at 50 °C overnight. The aqueous layer was extracted with ethyl acetate which was dried over Na_2SO_3 and reduced *in vacuo* to give compound **4.56** as a colourless oil; δ_H (400 MHz, $CDCl_3$) 8.04 (d, 1H, *J* 8, C*H*-CCOOH), 7.47 (t, 1H, *J* 7.5, C*H*-CH-CBu), 7.30-7.25 (m, 2H, C*H*-CBu and C*H*-CH-CCOOH), 3.04 (dd, 1H, *J* 8.5, 7.5, C*H*$_2$-aryl),

1.62 (quintet, 2H, *J* 7.5, C<u>*H*</u>$_2$-CH$_2$-aryl), 1.41 (sextet, 2H, *J* 7.5, C<u>*H*</u>$_2$-CH$_3$) and 0.95 (t, 3H, *J* 7.5, C<u>*H*</u>$_3$); δ_C (100 MHz, $CDCl_3$) 173.5 (<u>*C*</u>=O), 146.0 (<u>*C*</u>-Bu), 132.8 (<u>*C*</u>H-CH-CBu), 131.7 (<u>*C*</u>H-CCOOH), 131.3 (<u>*C*</u>H-CBu), 128.1 (<u>*C*</u>-COOH), 125.8 (<u>*C*</u>H-CH-CCOOH), 34.4 (<u>*C*</u>H$_2$-aryl), 34.0 (<u>*C*</u>H$_2$-CH$_2$-aryl), 22.8 (<u>*C*</u>H$_2$-CH$_3$) and 13.9 (<u>*C*</u>H$_3$); ESI *m/*z 100 %, 179.1 (MH^+) and 34 %, 201.1 (MNa^+).[250]

4.57, 3-Butylbenzoyl chloride

Acid **4.54** was dissolved in dichloromethane, oxalyl chloride and DMF (1 drop, catalytic) were added and the reaction was stirred for 3 hours. The organic solvents were removed *in vacuo* and compound **4.57** was used straight away in the next step.

4.58, 3-Hexylbenzoyl chloride

Acid **4.55** (0.50 g, 2.4 mmol) was dissolved in dichloromethane (25 mL), oxalyl chloride (0.4 mL, 4.8 mmol) and DMF (1 drop, catalytic) were added and the reaction was stirred for 3 hours. The organic solvents were removed *in vacuo* and compound **4.58** was used straight away in the next step.

4.59, 2-Butylbenzoyl chloride

Acid **4.56** was dissolved in dichloromethane (5 mL), oxalyl chloride (0.3 mL, 2 mmol) and DMF (1 drop, catalytic) were added and the reaction was stirred for 3 hours. The organic solvents were removed *in vacuo* and compound **4.59** was used straight away in the next step.

4.60, 3-Butylbenzoyl-(*S*)-3-amino-azepan-2-one

Lactam **2.65** (0.72 g, 4.39 mmol) was dissolved in H_2O (20 mL) and cooled to 0°C. Acid chloride **4.57** in dichloromethane was added and triethylamine (1.3 mL, 9

mmol) and the reaction was stirred over night. H_2O (20 mL) was added and the reaction was extracted with dichloromethane (3 × 20 mL), the organic layer was washed with a pH 2 buffer (3 × 15 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 75:25 to 0:100) to give compound **4.60** as a white solid 0.05 g (6 %); *mp* 105-106 º C; *Anal.* ($C_{10}H_{18}N_2O_2$ requires: C; 70.80, H; 8.39, N; 9.71) found: C; 70.68, H; 8.48, N; 9.68; δ_H (400 MHz, $CDCl_3$) 7.71-7.62 (m, 3H, Ar & N*H*-CH), 7.35-7.29 (m, 2H, Ar), 7.06 (br.t, 1H, *J* 6, N*H*-CH_2), 4.72 (dd, 1H, *J* 11, 6, lactam C*H*), 3.37-3.32 (m, 2H, lactam C*H*$_2$-NH), 2.65 (t, 2H, *J* 8, CH_2-aryl), 2.22 (br.d, 1H, *J* 13.5, equatorial lactam C*H*$_2$-CH), 2.03 (br.d, 1H, *J* 14, equatorial lactam C*H*$_2$-CH_2CH), 1.95-1.81 (m, 2H, equatorial lactam C*H*$_2$-CH_2NH and axial lactam C*H*$_2$-CH_2CH), 1.61 (quintet, 2H, *J* 7, C*H*$_2$-CH_2-aryl), 1.57-1.48 (m, 1H, axial lactam C*H*$_2$-CH), 1.46-1.36 (m, 1H, *J* 13, axial lactam C*H*$_2$-CH_2NH), 1.34 (sextet, H2, *J* 7.5, C*H*$_2$-CH_3) and 0.92 (t, 3H, *J* 7.5, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 176.0 (C=O), 166.6 (C=O), 143.4 (*C*-^{n}Bu), 134.2 (*C*-C=O), 128.4 (CH-*C*H-CBu), 127.2 (*C*H-CH-CBu), 126.8 (*C*H-CBu), 124.0 (*C*H-CCO), 52.6 (*C*H-NH), 42.1 (lactam *C*H_2-NH), 35.6 (lactam *C*H_2-CH), 33.5 (*C*H_2-aryl), 31.6 (*C*H_2-CH_2-aryl), 28.9 (lactam *C*H_2-CH_2CH), 28.0 (*C*H_2-CH_3), 22.3 (lactam *C*H_2-CH_2NH) and 13.9 (*C*H_3); υ_{max}/cm^{-1}: 3244 (NH), 2931 (C-H), 1648 (C=O) and 1559 (aromatic); ESI *m/*z 100 %, 598.8 (M_2Na^+) and 95 %, 311.2 (MNa^+); HR ESI *m/*z ($C_{17}H_{24}N_2O_2Na^+$ requires 311.1730) found 311.1725; $[\alpha]^{26}_D$ (c = 0.228, $CHCl_3$) +68.89.

4.61, 3-Hexylbenzoyl-(*S*)-3-amino-azepan-2-one

Lactam **2.65** (0.40 g, 2.4 mmol) was dissolved in H_2O (10 mL) and cooled to 0°C. Acid chloride **4.58** (0.56 g, 2.7 mmol) in dichloromethane (25 mL) and triethylamine

(1 mL, 8 mmol) were added and the reaction was stirred over night. H_2O (20 mL) was added and the reaction was extracted with dichloromethane (3 × 20 mL), the organic layer was washed with a pH 2 buffer (3 × 15 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 75:25 to 0:100) to give compound **4.61** as a white solid 0.45 g (53 %); *mp* 104-105 º C; $[\alpha]^{29}_D$ (c = 0.4, $CHCl_3$) +64.63; υ_{max}/cm^{-1}: 3203 (NH), 2925 (C-H), 1680, 1646 (C=O) and 1541 (aromatic); δ_H (400 MHz, $CDCl_3$) 7.65-7.60 (m, 3H, Ar & N*H*-CH), 7.35-7.29 (m, 2H, Ar), 6.27 (br.t, 1H, *J* 6, N*H*-CH_2), 4.71 (ddd, 1H, *J* 11.5, 5.5, 2, C*H*), 3.39-3.22 (m, 2H, lactam C*H*$_2$-NH), 2.63 (t, 2H, *J* 7.5, C*H*$_2$-aryl), 2.23 (br.d, 1H, *J* 12.5, equatorial lactam C*H*$_2$-CH), 2.08-2.00 (m, 1H, lactam C*H*$_2$-CH), 1.97-1.89 (m, 2H, lactam C*H*$_2$-CH_2NH and C*H*$_2$-CH_2CH), 1.65-1.50 (m, 2H, C*H*$_2$-CH_2-aryl), 1.42 (br.q, 1H, *J* 13.5, axial lactam C*H*$_2$-CH_2NH), 1.36-1.24 (m, 6H, C*H*$_2$-C*H*$_2$-C*H*$_2$-CH_3) and 0.86 (dd, 3H, *J* 7, 8, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 175.8 (lactam *C*=O), 166.5 (*C*=O), 143.5 (*C*-Hex), 134.2 (*C*-C=O), 131.7 (CH-*C*H-CHex), 128.4 (*C*H-CH-CHex), 127.1 (*C*H-CHex), 124.3 (*C*H-CCO), 52.6 (*C*H-NH), 42.3 (lactam *C*H_2-NH), 35.9 (lactam *C*H_2-CH), 35.9 (*C*H_2-aryl), 31.4 (*C*H_2-CH_2-aryl), 29.0 (lactam *C*H_2-CH_2CH), 28.9 (*C*H_2-CH_2CH_2-aryl), 28.0 (*C*H_2-CH_2CH_3), 22.6 (lactam *C*H_2-CH_2NH), 22.5 (*C*H_2-CH_3) and 14.1 (*C*H_3); ESI *m/*z 100 %, 339.2 (MNa^+) and 17 %, 654.6 (M_2Na^+); HR ESI *m/*z ($C_{19}H_{29}N_2O_2Na^+$ requires 317.2224) found 311.2227.

4.62, 2-Butylbenzoyl-(*S*)-3-amino-azepan-2-one

Lactam **2.65** (0.16 g, 1.0 mmol) was dissolved in H_2O (5 mL) and cooled to 0°C. Acid chloride **4.59** in dichloromethane (5 mL) and triethylamine (0.4 mL, 3 mmol) were added, the reaction was stirred over night. H_2O (10 mL) was added and the

reaction was extracted with dichloromethane (3 × 20 mL), the organic layer was washed with a pH 2 buffer (3 × 15 mL), dried over Na_2SO_4 and reduced *in vacuo*. The product was purified by silica column chromatography (petroleum ether:ethyl acetate 50:50 to 0:100) to give the product compound **4.62** as a white solid (0.08 g, 2 % over 4 steps); δ_H (400 MHz, $CDCl_3$) 7.37 (dd, 1H, C*H*-CCO), 7.31 (td, 1H, C*H*-CH-CBu), 7.23-7.15 (m, 3H, C*H*-CBu , C*H*-CH-CCO and N*H*-CH) 6.28 (br.t, 1H, *J* 5.5, N*H*-CH_2), 4.71 (ddd, 1H, *J* 11, 6, 1.5, lactam C*H*), 3.39-3.21 (m, 2H, lactam C*H*$_2$-NH), 2.80, 2.74 (dt, 1H, *J* 14, 8, CH_2-aryl), 2.57 (br.d, 1H, *J* 12, equatorial lactam C*H*$_2$-CH), 2.09-2.00 (m, 1H, equatorial lactam C*H*$_2$-CH_2CH), 1.97-1.83 (m, 2H, equatorial lactam C*H*$_2$-CH_2NH and axial lactam C*H*$_2$-CH_2CH), 1.62-1.50 (m, 3H, C*H*$_2$-CH_2-aryl and axial lactam C*H*$_2$-CH), 1.48-1.38 (m, 1H, *J* 13, axial lactam C*H*$_2$-CH_2NH), 1.34 (sextet, H2, *J* 7, C*H*$_2$-CH_3) and 0.89 (t, 3H, *J* 7.5, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 175.5 (*C*=O), 169.3 (*C*=O), 141.1 (*C*-^{n}Bu), 136.5 (*C*-C=O), 130.1 (*C*H-CH-CBu), 129.8 (*C*H-CBu or *C*H-CH-CO), 127.2 (*C*H-CCO), 125.7 (C *C*H-CBu or *C*H-CH-CO), 52.5 (*C*H-NH), 42.1 (lactam C*H*$_2$-NH), 33.0 (lactam *C*H_2-CH), 33.0 (*C*H_2-aryl), 31.6 (*C*H_2-CH_2-aryl), 29.0 (lactam CH_2-CH_2CH), 28.0 (*C*H_2-CH_3), 22.7 (lactam *C*H_2-CH_2NH) and 14.0 (*C*H_3); ESI *m/*z 100 %, 289.1 (MNa^+), 60 %, 311.1 (MNa^+) and 16 %, 598.6 (M_2Na^+); HR ESI *m/*z ($C_{17}H_{24}N_2O_2Na^+$ requires 311.1730) found 311.1733.

6.445 Iron-Cross Coupling on Amides

4.63, 4-Chlorobenzoyldimethylamide

4-(Chlorobenzoylchloride) (2.5 mL, 20 mmol) was dissolved in dichloromethane (25 mL) and cooled on ice. Dimethylamine (1.0 mL, 20 mmol) and triethylamine (8.4 mL, 60 mmol) and the reaction was stirred over night. The dichloromethane was

removed *in vacuo* and the product dissolved in ethyl acetate, washed with pH 2 buffer (3 × 10 mL), dried over Na_2SO_3 and reduced *in vacuo* to give compound **4.63** as a brown oil (2.28 g, 62 %); δ_H (400 MHz, $CDCl_3$) 7.23 (s, 4H, aryl) and 2.94, 2.82 (br.s, 3H, $N(C\underline{H}_3)_2$; δ_C (100 MHz, $CDCl_3$) 170.3 (C=O), 135.4 (<u>*C*</u>-Cl), 134.7 (<u>*C*</u>-CO), 128.6 (aryl <u>*C*</u>H) and 39.4, 35.3 ($N\underline{C}H_3$); ESI *m/*z 100 %, 206.0 (MH^+), 62 %, 388.8 (M_2Na^+) and 9 %, 284.2 (MH^+). Data is consistant with data previously reported for this compound.[251]

4.64, 4-Butylbenzoyldimethylamide

Compound **4.63** (1.04 g, 5.67 mmol) was dissolved in THF (20 mL) and cooled on ice. $Fe(acac)_3$ (0.20 g, 0.57 mmol) and NMP (3.3 mL, 34 mmol) were added. The reaction was stirred under nitrogen gas and HexMgCl (3.8 mL, 2M in THF) was added drop wise with a syringe. The reaction was stirred for 2 hours before quenching with pH 2 buffer and extracting with ethyl acetate (3 × 10 mL) and washing with pH 2 buffer (3 × 10 mL). The NMP was removed using silica column chromatography (petroleum ether:ethyl acetate 75:25) to give compound **4.64** as a light brown colourless oil (0.76 g, 58 %); δ_H (400 MHz, $CDCl_3$) 7.29 (d, 2H, *J* 8, C<u>*H*</u>-CO), 7.16 (d, 2H, *J* 8, C<u>*H*</u>-C-Bu), 3.06, 2.95 (br.s, 3H, $N(C\underline{H}_3)_2$), 2.58 (t, 2H, *J* 8, $C\underline{H}_2$-aryl), 1.57 (quintet, 2H, *J* 7.5, $C\underline{H}_2$-CH_2-aryl), 1.33-1.23 (m, 6H, $C\underline{H}_2$-$C\underline{H}_2$-$C\underline{H}_2$-CH_3) and 0.84 (dd, 3H, *J* 8.5, 7.5, $C\underline{H}_3$); δ_C (100 MHz, $CDCl_3$) 171.8 (C=O), 144.6 (<u>*C*</u>-Hex), 133.5 (<u>*C*</u>-CO), 128.3 (<u>*C*</u>H-C-Bu), 127.2 (<u>*C*</u>H-CO), 39.7 ($\underline{C}H_2$-aryl), 35.6 ($\underline{C}H_2$-CH_2-aryl), 31.5 ($\underline{C}H_2$-CH_2CH_2-aryl), 28.9 ($\underline{C}H_2$-CH_2CH_3), 22.6 ($\underline{C}H_2$-CH_3) and 14.1 ($\underline{C}H_3$); ESI *m/*z 100 %, 489.0 (M_2Na^+), 11 %, 256.1 (MNa^+), 7 %, 467.0 (M_2H^+) and 5 %, 234.2 (MH^+). Data is consistent with data previously reported for this compound.[249]

4.65, 4-Chlorobenzonyl-(*S*)-3-amino-azepan-2-one

4-(Chlorobenzoylchloride) (1.42 g, 9.07 mmol) was dissolved in dichloromethane (20 mL) and cooled on ice. Lactam **2.65** (1.38 g, 8.41 mmol) was dissolved in H_2O (15 mL) and added as was triethylamine (3.5 mL, 25.2 mmol) and the reaction was stirred over night. The reaction was extracted with dichloromethane (3 × 10 mL) and washed with pH 2 buffer (3 × 10 mL), dried over Na_2SO_3 and recued *in vacuo*. The product was purified by washing with hot ethyl acetate to give compound **4.65** as a white solid (0.46 g, 20 %); *mp* 217-218 °C; $[\alpha]^{31}_D$ (c = 0.592, MeOH) +0.318; υ_{max}/cm^{-1}: 3198 (NH), 2927 (C-H), 1660, 1641 (C=O) and 856 (C-Cl); δ_H (400 MHz, $CDCl_3$) 7.77 (d, 2H, *J* 8.5, C*H*-CCl), 7.61 (d, 1H, *J* 5.5, N*H*-CH), 7.40 (d, 1H, *J* 8.5, C*H*-CO), 6.08 (br.t, 1H, N*H*-CH_2), 4.68 (ddd, 1H, *J* 11, 6, 2, lactam C*H*), 3.39-3.24 (m, 2H, lactam C*H*$_2$-NH), 2.29 (br.d, 1H, *J* 14.5, lactam C*H*$_2$-CH), 2.10-2.01 (m, 1H, lactam C*H*$_2$-CH_2NH), 1.96-1.89 (m, 2H, lactam C*H*$_2$-CH_2CH), 1.61-1.50 (m, 1H, lactam C*H*$_2$-CH) and 1.43 (br.q, 1H, *J* 14, lactam C*H*$_2$-CH_2NH); δ_C (100 MHz, $CDCl_3$) 175.7 (lactam C=O), 165.2 (C=O), 137.8 (*C*-Cl), 132.6 (*C*-CO), 128.8 (*C*-CCl), 128.5 (*C*-CCO), 52.7 (*C*H), 42.2 (lactam *C*H_2-NH), 31.6 (lactam *C*H_2-CH), 28.9 (lactam *C*H_2-CH_2CH) and 28.0 (lactam *C*H_2-CH_2NH); ESI *m/*z 100 %, 289.0 (MNa^+) and 41 %, 554.6 (M_2Na^+); HR ESI *m/*z ($C_{13}H_{15}N_2O_2ClNa^+$ requires 289.0714) found 289.0718.

4.66, 4-Chlorobenzenesulphonyl-(*S*)-3-amino-azepan-2-one

4-(Chlorobenzenesulfonylchloride) (1.20 g, 5.69 mmol) was dissolved in dichloromethane (15 mL) and cooled on ice. Lactam **2.65** (0.96 g, 5.85 mmol) was dissolved in H_20 (10 mL) and added as was triethylamine (2.4 mL, 17.1 mmol) and the reaction was stirred over night. The reaction was extracted with dichloromethane

(3 × 10 mL) and washed with pH 2 buffer (3 × 10 mL), dried over Na_2SO_3 and recued *in vacuo*. The product was purified by washing with hot ethyl acetate to give compound **4.66** as a white solid; $[\alpha]^{28}_D$ (c = 0.52, $CHCl_3$) +123.56; δ_H (400 MHz, $CDCl_3$) 7.77 (d, 2H, *J* 8, C*H*-CCl), 7.45 (d, 1H, *J* 8, C*H*-CSO_2), 6.21 (d, 1H, *J* 5.5, N*H*-CH), 6.06 (br.t, 1H, *J* 4, N*H*-CH_2), 3.82 (ddd, 1H, *J* 11, 6, 2, C*H*), 3.23-3.14 (m, 1H, lactam C*H*$_2$-NH), 3.11-3.02 (m, 1H, lactam C*H*$_2$-NH), 2.23 (br.d, 1H, *J* 11, lactam C*H*$_2$-CH), 2.00 (br.d, 1H, *J* 13, lactam C*H*$_2$-CH_2CH), 1.80 (br.d, 1H, *J* 12, lactam C*H*$_2$-CH_2NH), 1.70-1.55 (m, 2H, lactam C*H*$_2$-CH_2CH and C*H*$_2$-NH) and 1.34 (br.q, 1H, *J* 14, lactam C*H*$_2$-CH_2NH); δ_C (100 MHz, $CDCl_3$) 174.0 (C=O), 139.2 (*C*-SO_2), 138.6 (*C*-Cl), 129.4 (*C*H-CSO_2), 128.5 (*C*H-CCl) 55.5 (*C*H), 42.3 (lactam *C*H_2-NH), 33.4 (lactam *C*H_2-CH), 28.6 (lactam *C*H_2-CH_2CH) and 28.0 (lactam *C*H_2-CH_2NH); ESI *m/*z 100 %, 325.0 (MNa^+) and 60 %, 626.7 (M_2Na^+); HR ESI *m/*z ($C_{12}H_{15}N_2SO_3ClNa^+$ requires 325.0384) found 325.0386.

4.67, 4-Chlorobenzonyl-(*S*)-3-amino-tetrahydropyridin-2-one

4-(Chlorobenzoylchloride) (13 mmol) was dissolved in dichloromethane (30 mL) and cooled on ice. Lactam **2.53** (10 mmol) was dissolved in H_2O (20 mL) and added as was triethylamine (4.2 mL, 30 mmol) and the reaction was stirred over night. The reaction was extracted with dichloromethane (3 × 15 mL) and washed with pH 2 buffer (3 × 15 mL), dried over Na_2SO_3 and recued *in vacuo*. The product was purified by washing with hot ethyl acetate to give compound **4.67** as a white solid (1.16 g, 46 %); *mp* 194-195 °C; υ_{max}/cm^{-1}: 3211 (NH), 2937 (C-H), 1670, 1649 (C=O) and 845 (C-Cl); δ_H (400 MHz, d^6 DMSO) 8.70 (d, 1H, *J* 8.5, N*H*-CH), 7.88 (d, 2H, *J* 8.5, C*H*-CCl), 7.66 (br.t, 1H, N*H*-CH_2), 7.53 (d, 1H, *J* 8.5, C*H*-CO), 4.37 (8, 1H, *J* 8.5, lactam C*H*), 3.19-3.14 (m, 2H, lactam C*H*$_2$-NH), 2.04-1.99 (m, 1H,

lactam C<u>*H*</u>$_2$-CH) and 1.87-1.74 (m, 2H, lactam C<u>*H*</u>$_2$-CH and lactam C<u>*H*</u>$_2$-CH$_2$NH); δ_C (100 MHz, d^6 DMSO) 169.7 (lactam C=O), 164.7 (C=O), 136.0 (<u>*C*</u>-Cl), 133.0 (<u>*C*</u>-CO), 129.2 (<u>*C*</u>H-CCl), 128.3 (<u>*C*</u>H-CCO), 49.6 (<u>*C*</u>H), 41.2 (lactam <u>*C*</u>H$_2$-NH), 27.6 (lactam <u>*C*</u>H$_2$-CH) and 21.3 (lactam <u>*C*</u>H$_2$-CH$_2$NH); ESI *m/*z 100 %, 275.0 (MNa^+) and 71 %, 526.6 (M_2Na^+); HR ESI *m/*z ($C_{12}H_{13}N_2O_2ClNa^+$ requires 275.0558) found 275.0554.

4.68, 4-Chlorobenzenesulphonyl-(*S*)-3-amino-tetrahydropyridin-2-one

4-(Chlorobenzenesulfonylchloride) (1.95 g, 9.24 mmol) was dissolved in dichloromethane (25 mL) and cooled on ice. Lactam **2.53** (10 mmol) was dissolved in H_2O (10 mL) and added as was triethylamine (8.4 mL, 60 mmol) and the reaction was stirred over night. The reaction was extracted with dichloromethane (3 × 10 mL) and washed with pH 2 buffer (3 × 10 mL), dried over Na_2SO_3 and reduced *in vacuo*. The product was purified by washing with hot ethyl acetate to give compound **4.68** as a white solid (1.11 g, 41 %); *mp* 136-137 °C; $[\alpha]^{30}_D$ (c = 0.526, $CHCl_3$) +123.42; υ_{max}/cm^{-1}: 3213, 3089 (NH), 2945 (saturated CH), 1655 (C=O), 1331, 1157 (SO_2), 827 (C-Cl); δ_H (400 MHz, $CDCl_3$) 7.81 (d, 2H, *J* 8.5, C<u>*H*</u>-CCl), 7.44 (d, 1H, *J* 8.5, C<u>*H*</u>-CSO$_2$), 6.55 (br.s, 1H, N<u>*H*</u>-CH$_2$), 6.19 (d, 1H, *J* 4, N<u>*H*</u>-CH), 3.52 (ddd, 1H, *J* 11, 6, 4, C<u>*H*</u>), 3.26-3.19 (m, 2H, lactam C<u>*H*</u>$_2$-NH), 2.39-2.30 (m, 1H, lactam C<u>*H*</u>$_2$-CH), 1.94-1.84 (m, 1H, lactam C<u>*H*</u>$_2$-CH$_2$CH) and 1.82-1.62 (m, 2H, lactam C<u>*H*</u>$_2$-CH$_2$CH and lactam C<u>*H*</u>$_2$-CH); δ_C (100 MHz, $CDCl_3$) 170.1 (C=O), 139.1 (<u>*C*</u>-SO$_2$), 138.2 (<u>*C*</u>-Cl), 129.7 (<u>*C*</u>H-CSO$_2$), 128.5 (<u>*C*</u>H-CCl), 53.3 (<u>*C*</u>H), 41.7 (lactam <u>*C*</u>H$_2$-NH), 28.6 (lactam <u>*C*</u>H$_2$-CH), and 20.7 (lactam <u>*C*</u>H$_2$-CH$_2$NH); ESI *m/*z 100 %, 311.0 (MNa^+) and 25 %, 598.4 (M_2Na^+); HR ESI *m/*z ($C_{11}H_{13}N_2SO_3Na^+$ requires 311.0228) found 311.0234.

6.6 Biological Testing

6.6.1 SSTR2 Binding Assay

The FP assay was run by Tilly Sharp or Glenda Chandler or Total Scientific (Babraham Research Campus, Cambridge). The test compounds are dissolved in an aqueous 4 % DMSO solution and are added to a 96-well plate along with unlabelled somatostatin. Somatostatin labelled with fluorescein isothiocyanate (SS14-FITC) was added, and finally the SSTR2 in phosphate buffer solution (PBS). The plate is shaken for one minute and left at room temperature for one hour, after which the FP data is collected.

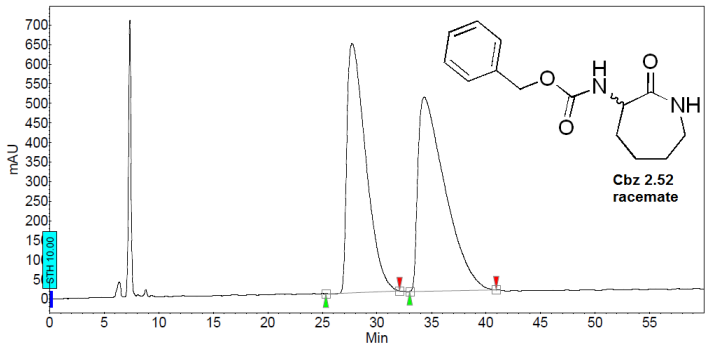
6.6.2 Leukocyte Migration Assay

The leukocyte migration assay was run by Dr Jill Reckless of The Department of Medicine, The University of Cambridge following a standard protocol.[200] A 96-well plate was used - each well with a lower compartment which contains the chemoattractant IL-8. The upper compartment contains the cells, which were neutrophils suspended in Gey's buffer. A polycarbonate filter membrane separates the compartments. The test compound was placed in each compartment at equal concentration, which was either 1 µM or 1 nM to ensure there was no concentration gradient. The experiments were randomised across the plate rather than spatially grouping the replications. The number of cells that migrated from the upper to the lower compartment was determined. The migrated cells were quantified using the dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The neutrophils were given 90 minutes to migrate after which 3µl of MTT (5mg/ml in Roswell Park Memorial Institute media [RPMI]) was added per well and the cells are incubated at 37 ˚C for 2 hours. A standard curve was set up with a known number of cells per well (in triplicate). After 2 hours the media was removed and the formazan

precipitate was solubilised with 20 µL DMSO. The absorbance at 595 nm was taken and the number of cells per well was interpolated from the standard curve of known number of cells.

6.7 Chromatograms

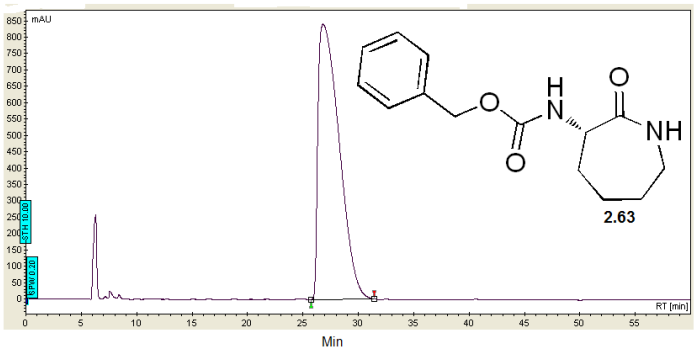
The optical purity was measured by HPLC analysis using a Varian Prostar 335 Photodiode Array Detector, using a Varian Prostar Solvent Delivery Module and a Varian Prostar 420 Autosampler. The *e.e.*s were determined using a CHIRALPAK AS column (4.6 mm × 250 mm) (*n*-hexane/propan-2-ol = 1:1; flow rate, 0.5 mL/min, λ = 207 nm).



Peak results :

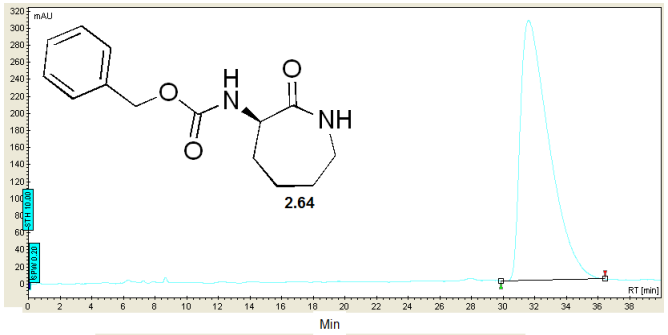
Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	27.70	47.21	637.4	1278.4	47.213
2	UNKNOWN	34.32	52.79	495.7	1429.3	52.787
Total			100.00	1133.2	2707.6	100.000

Figure 86 Chromatogram for Cbz protected lactam, pre-resolution compound **2.52**



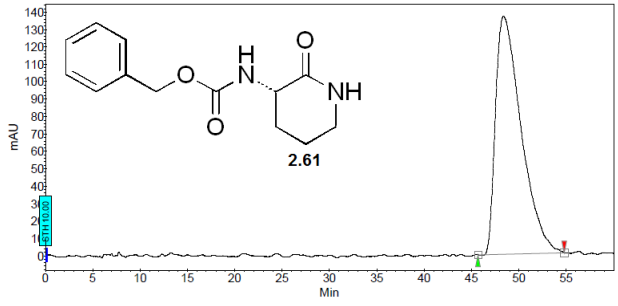
#	Name	Time [Min]	Quantity [% Are	Height [mAU]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	26.82	100.00	841.4	1860.6	100.000
Total			100.00	841.4	1860.6	100.000

Figure 87 Chromatogram for compound **2.63**



#	Name	Time [Min]	Quantity [% Are	Height [mAU]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	31.60	100.00	304.3	643.1	100.000
Total			100.00	304.3	643.1	100.000

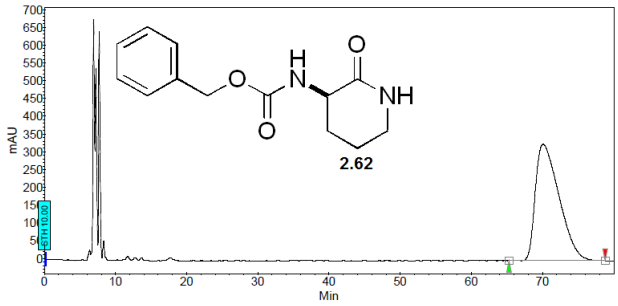
Figure 88 Chromatogram for compound **2.64**



Peak results :

Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	48.40	100.00	136.8	428.8	100.000
Total			100.00	136.8	428.8	100.000

Figure 89 Chromatogram for compound **2.61**



Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU Min]	Area % [%]
1	UNKNOWN	70.09	100.00	328.1	1261.4	100.000
Total			100.00	328.1	1261.4	100.000

Figure 90 Chromatogram for compound **2.62**

6.7 References

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